# STUDIES IN THE METABOLISM OF LEVULOSE

with special reference to

# A NEW FORM OF THE LEVULOSE TOLERANCE TEST.

by

Harold Scarborough.

From the Department of

Therapeutics.

14, June, 1937.

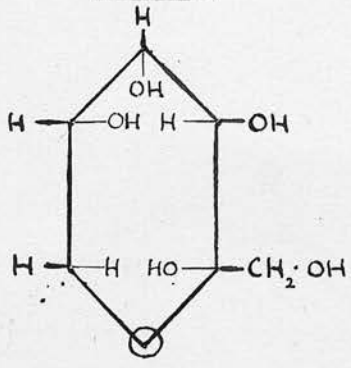
## FOREWORD:

The application of the procedure known as the levulose tolerance test to over a hundred normal and abnormal subjects has stimulated interest in the question: How is levulose metabolised?

The present essay contains a review of certain aspects of levulose metabolism and embodies also the results of levulose tolerance tests on normal and abnormal subjects. The withdrawal of blood samples has in nearly all cases been the work of the author. Mr. James Whittaker has been responsible for the majority of the blood sugar values and Dr. J. Munro has performed many of the large numbers of levulose estimations entailed.

It should be emphasised that this work is still in its early stages and is in no respect complete.

Part 1.




D.Levulose (fructo-pyranose) or fructose is a ketohexose isomeric with glucose. It is a levo-rotatory monosaccharide found fairly widely distributed in the vegetable world being present in honey, together with glucose, to the extent of 39 per cent. and in the juices of most sweet fruits especially tomatoes and mangoes but is not found as such in the animal body and cannot be regarded as having any important place in the diet of man.

Now although levulose in its free form is comparatively unimportant in the dietary, such quantities of the free sugar as are consumed forming a small and inconstant part of the diet, the disaccharide sucrose, as cane sugar (18-20 per cent.) or beet sugar (12-15 per cent.) is consumed in large and ever increasing quantities in this country and must be regarded as a normal article of diet. Sucrose (or Saccharose) is a dextrorotatory disaccharide /

disaccharide whose molecule is composed of a molecule of glucose and a molecule of fructose united through the potential aldehyde group with the elimination of a molecule of water. Fructose (levulose) is in fact prepared on a commercial scale by the inversion of sucrose and it may be of interest to note that the fructose used in experiments later to be described has been prepared according to the following method (Messrs. British Drug Houses, Ltd.) The juice of the sugar cane or beet is expressed and treated with milk of lime to neutralize acids and boiled to remove protein. The calcium is thereafter removed by $CO_2$ and the mixture decolorised with $SO_2$, after which it is again boiled, filtered and evaporated in vacuo until it crystallizes. The residue of crude molasses is boiled with more lime when an insoluble calcium saccharate separates out. This is decomposed by $CO_2$ and the solution on evaporation yields cane sugar. From this material fructose is obtained by hydrolysis with dilute acid the solution being subsequently neutralized by the addition of milk of lime. Fructose separates from the mixture as an insoluble calcium compound which is filtered off and decomposed by $CO_2$. Finally the solution is treated with charcoal to remove more coloring matter and impurities and evaporated to a syrup which can be dissolved /

dissolved in hot alcohol from which fructose slowly crystallizes.

At suitable pHs the enzyme invertase (or sucrase) rapidly hydrolyzes this sugar into an equimolecular mixture of Dd-glucose and Dl-fructose the process being widely known as the inversion of cane sugar and the product invert sugar. It has been shown repeatedly that the intestinal contents contain a sucrase whose optium pH is 6.8 (Euler and Svanberg). This enzyme in situ hydrolyzes sucrose very rapidly as witness the value of the administration of sucrose in diabetic hypoglycaemia. It is also a well established fact that dilute acids promote the hydrolysis of sucrose, the rate of hydrolysis depending to a large extent on the concentration of acid present. The concentration of the hydrochloric acid in the stomach, however, which is approximately N/10 (about pH 1.0) would have very little hydrolyzing effect on sucrose during the time for which the disaccharide is present in the stomach.

It has been stated that some commercial brown sugars contain invert sugar. If so, then this is another method by which small quantities of fructose in the free form might gain entrance to the body.

The polysaccharide inulin $(C_6H_{10}O_5)n$ which occurs in the roots of the dahlia plant (10-12 per cent.) the Jerusalem Artichoke, chickory, and many other plants of the order Compositae is a fructoside composed /

composed entirely of a large but unknown number of fructose units (probably 20 to 24. M.W.4000) united each to each with the elimination of water between each and on hydrolysis yields fructose only and no other monosaccharide. It is probable that the fructose present in inulin is y-fructose. (fructo-furanose). This form of fructose is more reactive than that possessing the formula given above and it is possible that it is this form of fructose which is metabolised in the body. The ordinary amylolytic enzymes in the alimentary tract have, however, no power to hydrolyze inulin and although a small part of the ingested inulin may possibly be hydrolyzed by the acid of the stomach, and a further quantity split by intestinal bacteria, it has been stated " that the value of inulin as a significant source of energy in human dietaries must be questioned." (Lewis - 1912).

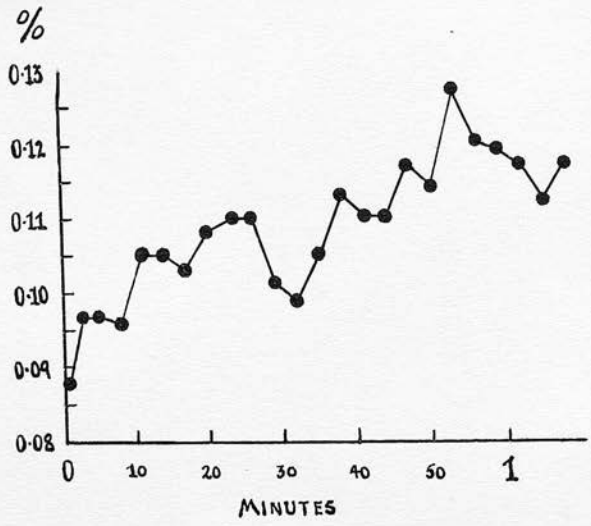
It is established, therefore, that the intestinal wall is normally concerned with the digestion and absorption of small quantities of levulose and such criticisms as are levelled against the administration of substances foreign to the body in order to test metabolic processes or the function of organs cannot with justification be applied to the administration of levulose. Furthermore it may be pointed out that although glucose has always been regarded as the 'physiological /

'physiological sugar', and lately galactose (as cerebroside) has been recognised to be an important constituent of the nervous system, fructose is now known to play an important part in the carbohydrate metabolism of muscle. It can in no wise be termed a "foreign" sugar.

If it be proposed, therefore, to investigate the metabolism of levulose and, or, to apply the procedure known as the levulose tolerance test hereinafter described, it is of paramount importance to have, in the first place, some information regarding the absorption by the intestinal wall of ingested levulose. It is just at this point that the first major difficulty arises for the precise mechanism of the absorption of this substance is not known.

From the application of physico-chemical considerations alone to this question, there can be no doubt that levulose is a substance capable of easy absorption by animal membranes. Since levulose and glucose are isomerides molar solutions of each (180.1 grammes per litre) should be absorbed by animal membranes at the same rate and no one doubts that ingested glucose is not absorbed with extreme rapidity. To demonstrate the rapidity of absorption of glucose, figures have been taken from a paper published /

Graph 1.



published by Hansen (1923) from which graph 1 has been constructed. Blood sugar estimations were made on capillary blood taken from the ear at three minute intervals by a stop watch and analyzed by the method of Hagedorn and Jensen. The glucose solution used contained 10 grams pure glucose in a 160 c.c. weak coffee and on analysis was found to be exactly 6 per cent. (5.4 per cent. glucose is isotonic with body fluids). The rise in blood sugar was found to be immediate (experimental error 1 per cent.). It is a matter of regret that present methods for the estimation of levulose in blood do not permit of similar experiments with ingested levulose in the human subject. Oppel (1929), however, detected levulose in the blood of rabbits within 5 minutes after the oral administration of this sugar in doses of 2.0 to 3.5 grams/kilo.

It has been suggested by Magee and Reid (1931) that the absorption of glucose from the intestine depends to some extent at any rate upon its phosphorylation, and the absorption of sugar is thus related to the metabolism of glucose by muscle. They found that the rate of absorption of glucose from the intestine of rats (Thiery-Vella fistulae) was accelerated by the addition of a phosphate buffer at pH7. /

pH7. This suggestion, however, has been criticised by Laszt (1935) who repeated these absorption experiments with an acetate buffer at pH7. Wilbrandt and Laszt (1932), however, showed that monoiodoacetic acid has a considerable inhibitory effect on the absorption of glucose and Verzar considers that this is due to the inhibition of phosphorylation of the sugar. Monoiodoacetic acid does not, however, prevent the phosphorylation of hexose,-Yamasaki (1930), Lohmann (1931) and Haarman (1932) (using bromo-acetic acid),-but in the opinion of Meyerhof (1935) inhibits the oxido-reductions from hexose diphosphate to glycerol phosphoric acids. It would seem, therefore, that if phosphorylation plays any part in the absorption of monosaccharides, the mechanism at work is analagous to the mechanism of fat absorption, i.e. a breakdown into simpler molecules with a resynthesis of hexose in the intestinal wall, since there is no evidence that portal blood during sugar absorption contains an increased concentration of phosphate. This idea receives support from the work of Laszt and Süllmann (1935) who have demonstrated an increase in acid-soluble organic phosphorus in the intestinal mucosa during hexose absorption from the intestine of rats. The intestinal contents would certainly form a suitable medium for hexose phosphorylation which could /

could not, of course, occur in the acid medium of the stomach.

If the absorption of glucose is dependent in part on its conversion in to phosphoric esters and if this process occurs normally in the upper part of the small intestine it might be concluded that the absorption of fructose involved a similar process. Verzar's experiments, however, would seem to show that phosphate plays a very much smaller part in the absorption of fructose and certainly one would not be justified in concluding that since the phosphorylation of glucose occurs during, or prior to, its absorption the same process occurs also with fructose, even though its molecule be closely similar, especially when it be considered that some breakdown of the glucose molecule may occur before its take-up by the intestinal wall.

There is no doubt that fructose is absorbed at a slower rate than glucose even when it is administered in the same concentration. The figures of Cori(1925) for the comparative rates of absorption of glucose and fructose (glucose 100, fructose 43) have been confirmed by a number of workers,the most recent of which is Verzar (1935) (glucose 100, fructose 44) who states that the faster absorption rates of glucose and /

Table 1.

Average vaules for the absorption of different Sugars before and after poisoning with moniodoacetic acid.

Expreased with normal absorption of glucose as 100.

| | Normal. | Poisoned. | Ratio. |
|---|---|---|---|
| Galactose | 115 | 53.1 | 2.1 |
| Glucose | 100 | 32.6 | 3.0 |
| Fructose | 44 | 36.8 | 1.2 |
| Mannose | 33 | 24.9 | 1.3 |
| Sorbose | 30 | 35.7 | 1.0 |
| Xylose | 30 | 30.5 | 1.0 |
| Arabinose | 29 | 28.8 | 1.0 |
| Rhamnose | 29 | 29.3 | 1.0 |

From Verzar "Absorption from the Intestine".

and galactose are due to a synthetic process involving phosphoric acid whereas the hexoses mannose and sorbose and the pentoses xylose, arabinose, and rhamnose are absorbed by pure diffusion. Verzar's results in this connection have been summarized in the form of a graph No. 2 and the effect of monoiodoacetic acid on relative rates of absorption is indicated in Table 1 (from Verzar "Absorption From The Intestine").

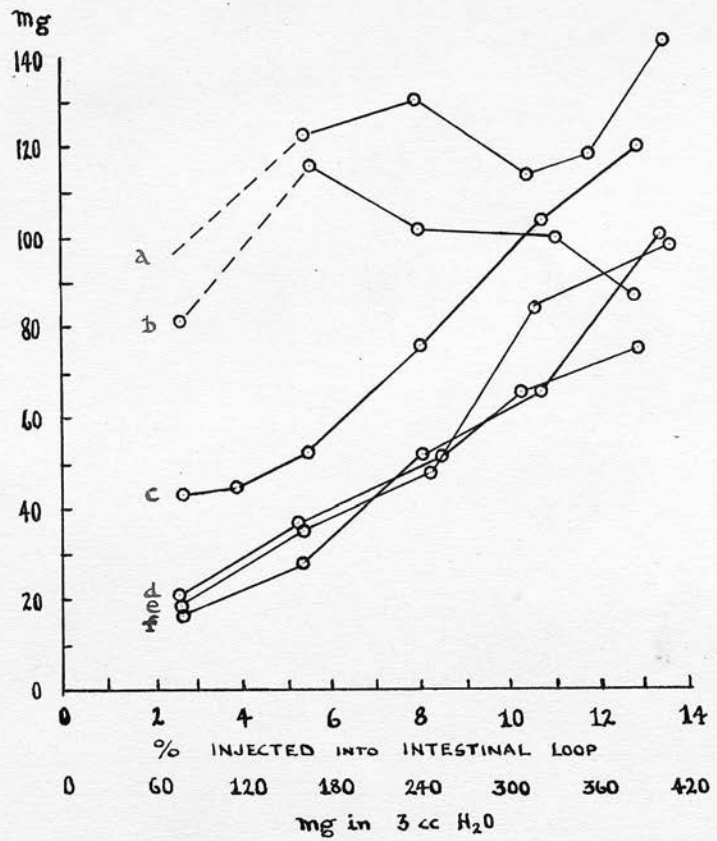
Table 1 shows quite clearly that there is a marked difference between the rates of absorption of galactose and glucose on the one hand and fructose on the other. It also strongly suggests that a different mechanism is at work in the case of fructose. While not necessarily agreeing with all Professor Verzar's conclusions, it would seem that the inhibition of selective absorption of glucose is not due to any general depression of membrane permeability since the rate of absorption of pentoses remains the same. Table 1 (from Verzar "Absorption From the Intestine") shows that the difference in the absorption rates of glucose and fructose is abolished by monoiodoacetic acid and contains the strong suggestion that the mechanism of fructose absorption differs from that of glucose. Fructose occupies an intermediate position in the table between glucose and pentoses and on this fact Verzar bases his idea that the fructose mechanism lies part way between the /

the mechanisms for the absorption of glucose and the pentoses. The suggestion here is that pentoses are absorbed by a process of pure diffusion, whereas glucose (and galactose) are absorbed partly by diffusion and partly by a process involving the formation of phosphorus esters, the latter process being abolished by monoiodoacetic acid. If this conclusion be drawn then phosphorylation must play a small part in the absorption of fructose (and mannose) or these sugars must be in part converted into glucose in the intestine. These experiments were, however, performed on rats and the number of rats used in the experiments is not stated, so that one has no means of assessing whether or not the changes in fructose values are significant. Certainly the inhibition of selective absorption of glucose is not due to any general depression of membrane permeability since the rate of absorption of pentoses remains the same both before and after monoiodoacetic acid.

Now if the absorption of glucose involves to any important extent the formation of phosphoric esters and its partial breakdown into simpler substances, an enzymic reaction is presumably involved and increasing concentration of glucose beyond a certain point should not increase the rate of absorption, the rate of the reaction being limited by the amount of enzyme present.

Cori /

GRAPH 2.




Relation of absolute absorption to concentration given of different sugars.

(a) glucose; (b) galactose; (c) fructose;
(d) xylose; (e) sorbose; (f) mannose;

From Verzar "Absorption from the Intestine."

Cori (1925) in a beautifully designed series of experiments showed that absorption of glucose from the intestine cannot surpass a certain maximum and that increasing doses of glucose beyond this level prolong the resultant hyperglycaemia without any further increase in the blood sugar level. The same thing was also shown for levulose. The relation of absolute absorption to concentration of sugar solution ingested has been determined graphically by Verzar (1935) who estimated the absorption of sugar from isolated intestinal loops in rats. Graph 2. From this graph it may be concluded first that a different absorption mechanism is at work in the case of glucose and galactose from that involved in the absorption of pentoses, and secondly that the absorption of these hexoses is not dependent on their concentration in the intestine after a concentration of 5 per cent. has been reached. The fact that glucose solution is approximately isotonic at this concentration might perhaps be noted in passing. The rate of absorption of mannose and the pentoses increases with their concentration, a result which would favour the conclusion that these are absorbed by a process of pure diffusion. The curve for fructose follows an intermediate course, but resembles in type the pentose curves. Again, one cannot agree with /

with Professor Verzar's conclusions entirely. The figures would appear to indicate that fructose is more readily absorbed than the pentoses and less readily than glucose and galactose, and that increasing concentrations of fructose in the gut determine an increased absorption of fructose by a process of pure diffusion.

With regard to the conversion of fructose into glucose, de Bruyn and van Eckenstein (1896) showed that under the influence of hydroxyl ions solutions of pure fructose were slowly transformed into a mixture of glucose, fructose and mannose up to an equilibrium point. The reaction, glucose⇌fructose⇌mannose, however, takes place only slowly in dilute alkaline solution and Neuberg and Leibowitz (1928) consider that conditions in the body are unlikely to favour the reaction. Rohmann (1915-19) and Kumagai (1913-14) have obtained evidence for the existence of stereo-kinases in the body and Isaac (1913) by perfusion experiments demonstrated that the liver produced glucose from fructose, the glycogen content of the liver meanwhile remaining constant. This result receives confirmation from Embden's laboratory. It is well established that fructose can, after absorption, increase the blood glucose level, but, as this /

this conversion may possibly take place through the medium of glycogen, it cannot be called as evidence for the direct transformation of fructose to glucose. Bollmann and Mann (1931), two careful workers, performed experiments on hepatectomised depancreatised dogs which apparently demonstrated that injections of fructose into the intestine raised the level of blood glucose and the glycogen content of muscle. Further, the intravenous injection of levulose prevented hypoglycaemia in hepatectomised dogs only if the alimentary tract and its blood supply remained intact. They concluded, therefore, that the intestinal wall has the power of converting fructose into glucose. Several attempts have been made to support this conclusion, - Berget, Moore and Lloyd, (1932) Oppel (1929) - but so far without success. It should be pointed out, perhaps, that the metabolic processes of Bollmann and Mann's animals could hardly be considered normal and furthermore that the influence of anaesthetics, especially ether, on absorption from the intestine is very considerable.

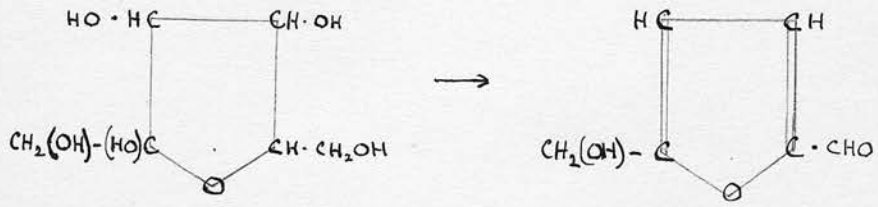
In order to throw further light on this matter Verzar has very recently devised an ingenious experiment which he terms " diffusion through double membranes" . A solution of fructose was enclosed with an extract of intestinal mucosa between two parchment membranes and the surrounding Ringer's solution /

solution analysed for fructose and glucose by the Bertrand and Willstaetter method. Results with this apparatus are said to show that about one-sixth of the fructose is converted into glucose and that this conversion is enzymic and occurs at pHs near neutrality.

It has been shown then, that the intestinal wall is normally accustomed to dealing with small quantities of levulose ingested either in the free form or as sucrose. The rate of absorption of levulose is only about 45 per cent. that of glucose, whereas mannose and the pentoses are absorbed at only 30 per cent. of the glucose rate. The selective action in respect of glucose has been ascribed to the formation of phosphorus esters and it is possible that fructose may in part be phosphorylated or transformed to glucose and then phosphorylated prior to absorption, and in part absorbed by a process of pure diffusion as are the pentoses. Perhaps it should be pointed out in this respect that conclusions drawn from experiments on human subjects directed towards determining the blood fructose level after ingestion of levulose are dangerous, since, after its absorption, at least three important factors, the liver, the kidney and the tissues /

tissues are concerned with the removal of fructose from the blood. One cannot do better than conclude with Deuel (1936) " further experimental evidence on the effect of concentration of various sugars on their relative absorption rates is needed."

It has been known for many years that the ingestion of large quantities of levulose produces the excretion of the unchanged sugar in the urine, a result which could only be produced if the sugar were carried unchanged in the blood from the intestine to the kidney and, therefore, analytical methods were sought for the estimation of levulose in blood and urine. The reaction which was first used for the detection of fructose was the Seliwanoff reaction (1887) which depends upon the conversion of fructose by hydrochloric acid into hydroxy-methyl-furfural (and levulinic acid).




The furfural then condenses with resorcinol to give a red compound. In order to perform quantitative experiments the red compound is extracted by alcohol producing a colored solution which can be compared colorimetrically with a standard. This quantitative procedure /

procedure has been used by Folin and Berglund (1922) and by Kronenberger and Rądt (1927). There are, however, several objections to the method. In the first place blood, and especially urine, contains interfering substances in the presence of which the reaction is no longer quantitative, and it has been found impossible to remove these substances completely. The reaction is not, in fact, specific, for fructose but is given by any keto-hexose and also, even under similar conditions, by glucose. In addition, pentoses interfere with the color produced. Furthermore, the brownish precipitate which is frequently formed, particularly when biological fluids are used, interferes considerably but irregularly with the color comparison and the color extraction by alcohol is not always complete. As a result of these objections the method was abandoned by Kronenberger and Radt and remained in desuetude until Roe (1934) investigated the matter again. By increasing the concentration of HCl from 12% to 18%, by using pure anhydrous ethyl alcohol, and by eliminating the reaction with glucose by heating at $80_0$C Roe states that the Seliwanoff procedure can be made superior to any other method for the estimation of fructose provided that a zinc hydroxide method be used for the removal of proteins. Even with these precautions it /

it is evident from Roe's paper that the very greatest care must be taken in carrying out this procedure - such as, for instance, that all tubes must be put into, and removed from, the water bath simultaneously by a stop watch. Extreme care is also necessary with the preparation and measuring of reagents, and, even so, the presence of pentoses interferes with the color produced. Considering all the evidence available it must be concluded that the Seliwanoff reaction is unsuitable for the quantitative estimation of fructose as a routine procedure and we have accordingly only employed it qualitatively for the detection of fructose in urine.

Campbell and Hanna (1926) devised a method based upon the reduction of molybdenum in phosphoric acid and the re-oxidation of the reduced molybdenum with $KMnO_4$. This reaction has been unfavourably reported on by Davidson et. al. (1936) who found that it did not go to completion. It is not specific for fructose, is not sensitive enough for biological work, and is time consuming, the time required for the molybdenum reduction being 90 minutes. It has now been entirely given up.

Scott (1935) evolved a method for the estimation of fructose based upon the Pettenkofer reaction. He states that the production of the reddish-purple color formed as a result of the reaction of fructose with /

with bile salt (sodium taurocholate) is proportional to the amount of fructose in solution. This reaction depends, however, upon the formation of furfural from fructose by the action of concentrated acid and, therefore, similar criticisms can be levelled against it as have been mentioned under the discussion of the Seliwanoff reaction.

The most satisfactory method for the quantitative determination of fructose in blood appears to be one based on the original method of van Creveld (1927) which employs the blue color produced when fructose reacts with diphenylamine in the presence of HCl, a reaction first described by Ihl and Pechmann (1885). In the original method proteins were precipitated by mercuric chloride but it was pointed out by Paterson (1935) that the filtrate contained substances which interfered with the color change, and, therefore, a zinc-hydroxide method of deproteinization was substituted. Paterson further found that the addition of $(NH_4)_2SO_4$ greatly facilitated the extraction of the blue color by butyl alcohol. We have ourselves employed the diphenylamine method of van Creveld in the form modified by Radt (1928) and Paterson (1935) and have introduced one further refinement, namely the drying of the butyl alcohol extract by sodium sulphate which often considerably facilitates /

facilitates comparison. In place of a colorimeter we employ a Pulfrich step-photometer previously calibrated from solutions of pure glucose-free A.R. fructose (B.D.H.) dissolved in blood filtrate. The best results were obtained with a filter S53 and a stratum thickness of 20 mm. (19.98).

The advantages of this method are considerable. Corley (1929) showed that glucose galactose and pentose (xylose, d- and l- arabinose) produced only 3 per cent of the colour development of fructose. We have found that ascorbic acid and glutathione will produce a blue colour with diphenylamine only when present in concentrations of the order of 15 - 25 mgms. per 100c.c. and the color production even then is very slight (estimating in terms of fructose as 4-6 mgms. per 100 c.c.) A whole series of estimations can be performed within an hour. The quantity of blood normally used for each estimation is only 2 c.c. but Davidson et. al. (1936) modified the procedure for 0.2 c.c. capillary blood using a colorimeter for comparisons. Recently we have worked out a method for the estimations of glucose, fructose, and "total blood sugar" requiring in all only 0.2 c.c. capillary blood. The fructose is estimated by the diphenylamine method using micro-cups on the step-photometer, the total reducing sugar by the method of Hagedorn and Jensen and the glucose obtained by difference. That this procedure is justifiable is indicated by the

work /

work of Davidson et. al. (1936) who showed that in the above technique fructose and glucose have the same reducing power.

The actual details of the method as used here are as follows:-

2 ml. of blood were pipetted into a boiling tube containing 14 ml. of water. 2 ml. 10 per cent. zinc sulphate solution and 2 ml. 0.5 N. sodium hydroxide were added. After thorough mixing of the contents, the tube was heated in a water-bath at $80_0$ for five minutes. After cooling, the mixture was filtered through a 9 cm. paper. 10 ml. of the filtrate were acidified with two drops of 1 per cent. acetic acid, and were evaporated, by free boiling, in a test-tube graduated at 4 ml. to just under the mark. The residual solution was then made up exactly to 4 ml. of 6 N.HCl and 0.1 ml. of 20 per cent. alcoholic diphenylamine were then added, and the tube, after shaking, was placed in a briskly boiling water-bath for fifteen minutes. After cooling, 10 ml. of butyl alcohol and 2 grams of solid ammonium sulphate were added. The tube was stoppered and briskly shaken. The upper alcoholic layer was pipetted off into a centrifuge tube, about 20 mgrms. anhydrous sodium sulphate were added, and, after shaking, the liquid was centrifuged for five minutes. The drying with sodium sulphate and the centrifuging made the comparison of unknown and standard in the colorimeter very much easier: when using the photometer it was essential, since the turbidity due to varying amounts of moisture in different analyses then made it impossible to measure accurately the absorption due to the colour.

It must be noted that estimations of fructose on the fasting blood of normal and abnormal subjects frequently give values of between 0 and 8 mgms. fructose per 100 c.c.s. The latter value is rarely encountered, the average for the blank being 3-4 mgms. per 100 c.c. It has been tacitly assumed by the various /

Table 2.

Effect of addition of potassium oxalate to blank readings.

Oxalate added to blood before protein precipitation.

| | | | |
|---|---|---|---|
| 2 | mg. in 2 c.c. | photometer reading | 0 |
| 20 | do. | do. | 0 |
| 100 | do. | do. | 2 |
| 500 | do. | do. | 15 |
| 1000 | do. | do. | 22 |

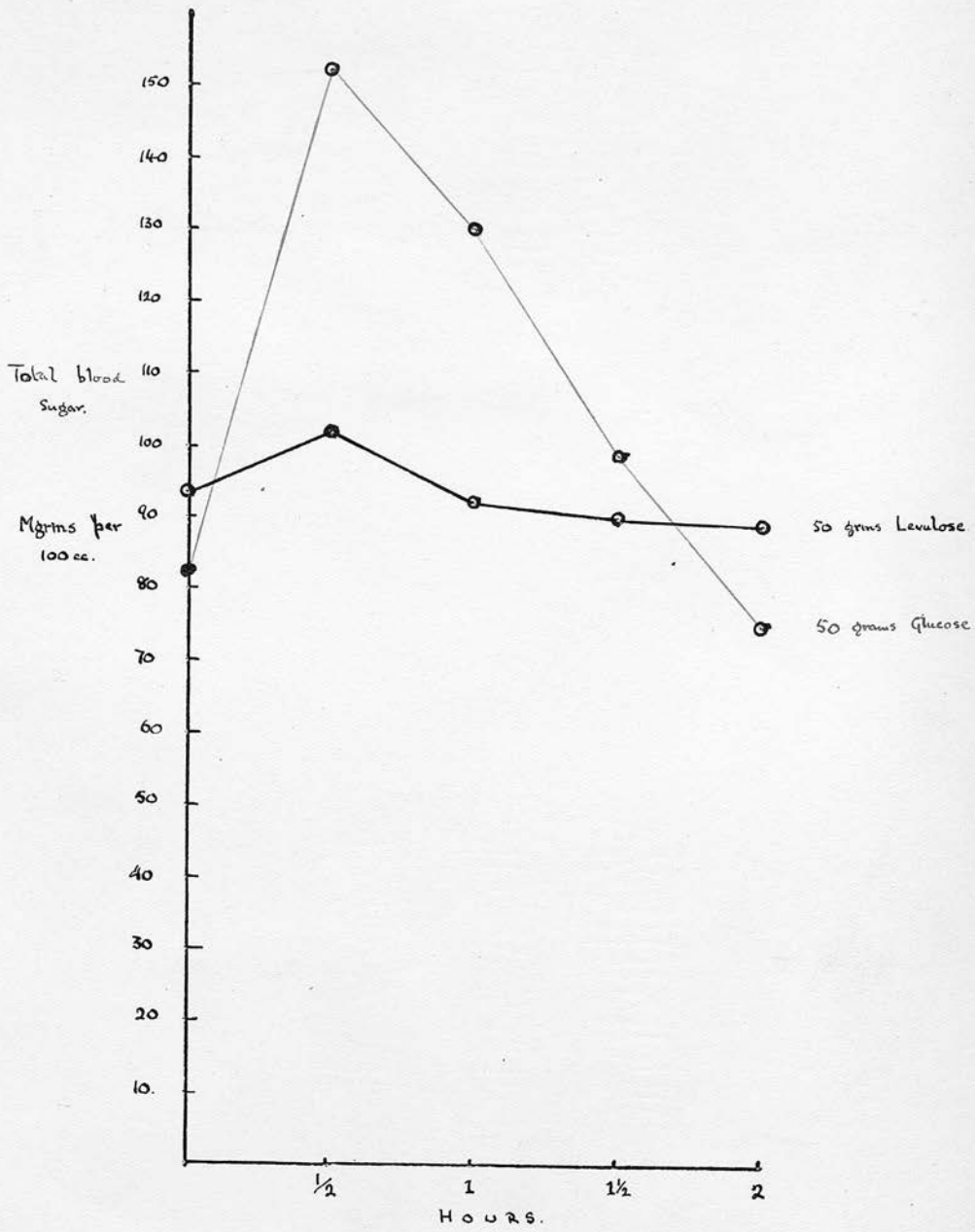
filt
not cl

various workers in this field that blood normally contains no fructose.

Adopting this assumption we carried out experiments to determine the nature of the blank, and, although these experiments are still in progress, we can now say that the addition of excessive amounts of oxalate to blood will account for an increase in the blank value. Table 2. shows the kind of results produced. Addition of large quantities of oxalate to the blood filtrate after precipitation of proteins, or to defibrinated blood, does not affect the final result. As the question is still under investigation it would perhaps be inadvisable to speculate as to further explanations for this state of affairs, suffice it to say, however, that we have not thought it proper to subtract the blank value from subsequent fructose readings on blood from the same subject since the properties of the photometer calibration curve make it evident that only at low fructose values (0 to 5 mgms. per 100 c.c.) does this difficulty arise. Using known solutions of fructose made up in blood filtrate the method is accurate to within 1 - 2 mgms. per 100 c.c. at all concentrations used.

We believe this method to be a distinct advance over any that has previously been employed and in view of the accuracy and precision afforded we consider that /

Graph 8.


Total blood Sugar.
Mgrms per 100 cc.
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10.
½
1
1½
2
HOURS.
50 grms Levulose
50 grams Glucose

that it is now possible to examine certain problems in levulose metabolism with an exactitude hitherto impossible.

Since we now possess an accurate method for the determination of levulose in blood it becomes possible to estimate the changes in blood fructose following the ingestion of a quantity of the sugar, and it is, therefore, necessary to enquire what possible factors there are which influence the amount of fructose in the blood at any one time. Absorption having been already dealt with, the functions of the liver, kidneys and tissues in this respect deserve brief consideration.

Following Folin and Berglund (1922) most observers agree that the rise in total blood sugar after the administration of fructose is considerably less than that caused by an equal amount of glucose. The sort of result obtained is indicated in graph, No.3. This difference was ascribed by Folin and Berglund to the more rapid uptake of fructose by the tissues, a conclusion which more modern methods of analysis have made untenable. Reinhold and Karr (1927) considered that fructose was more rapidly converted into glycogen by the liver than was glucose and also that the rate of oxidation in the tissues was possibly greater. Cori (1925) held that the difference was due to the slower absorption rate of fructose while Joliffe (1930-31) drew attention to the fact that the more rapidly a sugar is absorbed the more strain is thrown upon the /

the glycogenesis mechanisms and postulated that the higher blood sugars after glucose and galactose are due to the fact that absorption has over-run glycogenesis whereas with a more slowly absorbed sugar like fructose glycogenesis is able to proceed almost as rapidly as absorption.

Now if, after the ingestion of fructose, the blood levels of fructose, total sugar, and glucose be observed, then an explanation of the phenomenon is at once apparent. The lower blood sugar values after fructose ingestion are due to a fall in blood glucose. Why does the glucose level fall in this way? Davidson, Stewart and others (1936) employing on rabbits the analytical methods we have used, obtained excellent evidence that fructose is a better stimulus to insulin production by the pancreas than is glucose and hence a fall in blood glucose occurs after the ingestion and absorption of fructose.

A large number of observers following Ishmori (1913) have shown that fructose is converted into glycogen in the liver, and Cori (1925) showed that the rate of increase of liver glycogen was greater after fructose than after glucose. These results can now be interpreted in the light of Davidson's observations, though it should be noted that Bertram (1929) found a better glycogen formation with fructose even in the absence of insulin.

Corley /

Corley (1929) and Wierzuchowski (1926) both found that insulin had no effect on the rate of removal of fructose from blood. It has been shown repeatedly that the intravenous injection of fructose can both relieve hypoglycaemia in animals, although taking longer to do so than glucose, and also protect animals from the effect of excessive insulin dosage. Moreover, Bollman and Mann (1931) noted the conversion of injected fructose into glucose after extirpation of the pancreas. It would seem, therefore, that there are at least two stages in the metabolism of fructose, the first being the conversion of fructose into glucose, and the second the utilisation of the glucose so formed, only the second of these processes requiring the mediation of insulin. This suggestion receives the support from the work of Davidson et. al. (1936).

From the experiments of Mann and Magath (1922) who showed that the intravenous injection of fructose was unable to prevent hypoglycaemia convulsions in moribund hepatectomised dogs, whereas glucose could do so, and from the work of Bollman and Mann (1930) on the rates of removal of injected fructose from blood before and after hepatectomy, it can safely be concluded that the principal site for the conversion of fructose into glucose is the liver. This view receives abundant support from the work of Isaac (1920) and Kimball (1932) who demonstrated the prolonged /

prolonged fructosaemia in hepatic disease, and is, in fact, the basis for the so-called levulose tolerance test.

That it is not the only site for this conversion is suggested by experiments of Bollman and Mann to which reference has already been made.

Can the tissues utilize fructose directly or do they depend upon its previous transformation into glucose? Cori and Cori(1928), studying the mode of disposal of various sugars in rats, concluded that, of the total quantity of levulose absorbed from the alimentary tract in four hours, 36% was oxidised directly, 38% converted into liver glycogen and 12% into tissue glycogen.

Steinberg (1927) from studies of carbohydrate utilisation in isolated surviving tissues demonstrated that skeletal muscle was able to utilise fructose directly. This result supports McGuigan (1908) and is confirmed by Griesbach (1929). Scott (1935) using the bile salt method has demonstrated in normal and diabetic subjects an arterio-venous levulose difference of the order of 4-6 mgms. per 100 c.c. We have not yet been able to apply the diphenylamine method to the problem but this work is in immediate prospect.

McLean and Smedley (1912) and Stewart and Gaddie (1934) were unable to obtain any utilisation of fructose by the heart muscle of the dog, rabbit or frog. /

frog. Ashford (1933) reports a similar negative result for brain tissue. In the meantime, therefore, we feel that it would be well to keep an open mind on this question and to await further experimental evidence, remembering, however, that, since the first step in the metabolism of glucose is its conversion into a fructose diphosphoric acid, it would seem likely that fructose would be even more readily used in this reaction, especially in conditions of glycogen depletion.

Reference must also be made to experiments on the effect of fructose upon the R.Q. It has been quite definitely established that the rise in R.Q. following fructose ingestion occurs very much more promptly than after glucose. This was first shown by Tögel, Brezina and Durig (1913) and has been repeatedly confirmed. Various explanations have been offered for this phenomenon. We mention only the suggestion of Higgins (1916) which is supported by Cathcart and Markowitz (1927) that the higher R.Q. after fructose is due to a greater rate of fat formation rather than to a preferential combustion. Now, the alteration in R.Q. occurs within a few minutes after the ingestion of fructose suggesting to many observers that it is not indicative of a true metabolic process. Several workers, of whom may be mentioned Campbell and Maltby (1928) working on human subjects, have noted a rise in the blood lactic acid content with a coincident fall /

fall in the $CO_2$ combining power immediately after the ingestion of fructose and this suggests at once that the rise in the R.Q. so widely reported is accounted for by an increased excretion of $CO_2$. Apparently there is growing experimental support for this view, although Carpenter and Lee (1933) from careful experiments on the R.Q. of alveolar air conclude that the resultant acidaemia is not sufficient to account for the elevation of the R.Q. after fructose. Finally it must be observed that all this experimental data must be considered anew in the light of the experiments of Davidson et. al. and ourselves which lend strong support to the suggestion that the output of insulin is immediately increased after the ingestion of the fructose and that, therefore, the increased combustion of glucose may account simultaneously for the raised R.Q. and the increase in blood lactic acid. Simultaneous observations on the R.Q., $CO_2$ - combining power, and blood lactic acid after fructose and glucose ingestion might be of great significance in this respect and we hope to undertake work of this kind in the future.

The presence of glycosuria after the ingestion or injection of levulose has been known for many years. Methods for the estimation of fructose in urine, are, however, still unsatisfactory since what has been said with regard to the evaluation of fructose in blood applies /

applies with much greater force in the case of urine, and, therefore, as a result of the lack of precise method for its estimation, the question of a renal threshold for fructose is still to some extent a vexed one. The latest work on the subject is that of Roe (1934) which seems to indicate that the renal threshold for fructose is either low or non-existent. Older experiments of Folin and Berglund (1922) and of Bodanksy (1923) would suggest that the kidney possesses a definite threshold for fructose, so that in the absence of a thoroughly satisfactory analytical method the question must remain in doubt, though the conclusions of Roe would appear to be the better substantiated - Tashiro and Tietz (1930), Harding and Selby (1931). A possible analytical advance has recently been made in this direction, Deuel and Chambers (1925), and we hope to be able to modify the diphenylamine method to the estimation of urinary levulose.

In the preceding pages of this essay we have shown how levulose may normally gain entrance to the body and have considered in brief the mechanism of its absorption from the intestine. After reviewing the various methods available for its estimation in blood we have selected one which in its modified form is a considerable advance on any that has hitherto been used, and we have considered what is known regarding the removal of ingested levulose from blood and the possible mechanisms involved /

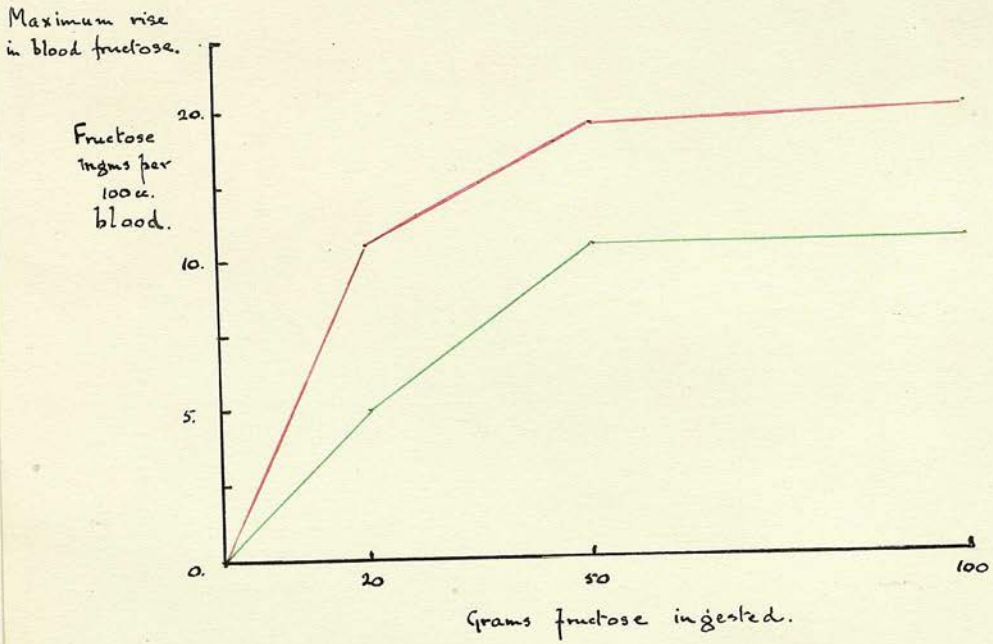
involved in this process. The second part of this work is devoted to a consideration of the normal response to levulose ingestion and to such deviations from the normal as are determined by disease using the procedure known as the levulose tolerance test but in a new and improved form involving the simultaneous determination of "total blood sugar" and blood levulose from which we are able to determine by subtraction the actual amount of glucose in the blood (plus the small amount of non-carbohydrate reducing substance not removed in the precipitation of blood protein). The history of the levulose tolerance test and a review of the results of other observers using methods less precise has been outlined by Stewart, Scarborough and Davidson (1937).

## Part II.

### The Administration of Levulose.

Folin and Berglund (1922) drew attention to the occurrence of alimentary disturbances of a more or less severe nature after the administration of levulose, attributing such effects to impurities existing in the sugar solution. Again Hansen (1923) noted headache, vertigo and nausea after the administration of pure glucose. These symptoms were common when the doses given were over 200 grams but occurred also in some individuals even with the smallest doses used, 20 grams. We ourselves have noted toxic symptoms - nausea, vomiting and vasomotor disturbances after doses of 50 grams of allegedly pure levulose in a high proportion of cases if particular precautions be not taken in its administration. It is important to use pure specimens of the sugar. Those obtained from B.D.H. prepared by the purification of B.P. levulose from the inversion of sucrose are satisfactory. 50 grams of the sugar are dissolved in 300 c.c. of boiling water, the juice of half a lemon is added and the drink is placed in a refrigerator until ice-cold. This latter precaution is important since it removes the nauseating 'burnt caramel' taste of levulose in such concentrated solution. The taking of this solution is preferably followed /

Graph 4.


Maximum rise in blood fructose.
Fructose mgms per 100 c.c. blood.
20.
10.
5.
0.
20
50
100
Grams fructose ingested.

followed by the drinking of 200 c.c. of cold water. Since taking these precautions we have not met unpleasant symptoms in any case even though we have performed the test on patients too ill to take an ordinary diet.

As to the selection of the dose of levulose, we have been concerned to keep this as low as possible yet capable of taxing the fructose removal mechanisms to the maximum. Graph 4. shows that 50 grams fulfills these conditions: its selection also allows comparison of our own results with the results of previous workers in this field who mainly used a 50 gram dose, though for no apparent particular reason.

All subjects used had fasted for at least 12 hours prior to the test. A specimen of blood (4 c.c.) was taken in the fasting state, the levulose at once administered, and, therefore, 4 c.c. specimens of blood withdrawn half an hour, one hour, 1½ hours, and two hours after ingestion, although lately we have usually omitted the 1½ hour specimen. As previously described, oxalation of the blood requires care.

The normal Response to Levulose Ingestion.

Using the technique described above, we have obtained the following results in a series of twenty normal healthy adults whose ages vary from 20 to 40. Cases in the hospital even though convalescent have not /

Graph 5.

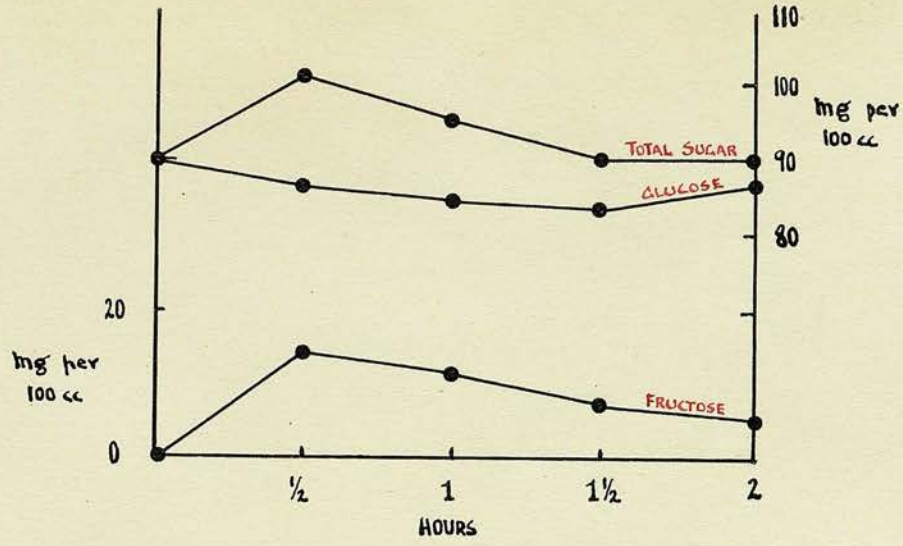



Table 3.

Normal Subjects.

| Subject. | Mgms. per 100 c.c. whole blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| | Hours after ingestion. | | | | | Hours after ingestion. | | | | | Hours after ingestion. | | | | |
| | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 3. | 83 | 93 | 84 | 81 | 80 | 0 | 12 | 13 | 10 | 5 | 82 | 81 | 71 | 71 | 75 |
| 4. | 93 | 102 | 92 | 90 | 89 | 0 | 8.5 | 10 | 12.5 | 4 | 93 | 93.5 | 82 | 77.5 | 85 |
| 6. | 82 | 91 | 84 | 82 | 80 | 0 | 14 | 10 | 8.5 | 3 | 82 | 77 | 74 | 73.5 | 77 |
| 7. | 90 | 92 | 85 | 83 | 82 | 0 | 18 | 13 | 7 | 4 | 90 | 74 | 72 | 76 | 78 |
| 10. | 99 | 108 | 100 | 98 | 98 | 0 | 16 | 14 | 12 | 3 | 99 | 92 | 86 | 86 | 95 |
| 11. | 90 | 94 | 92 | 90 | 90 | 0 | 15 | 14 | 11 | 5 | 90 | 79 | 78 | 79 | 85 |
| 12. | 90 | 102 | 96 | 90 | 90 | 0 | 14 | 11 | 8 | 4 | 90 | 88 | 85 | 82 | 86 |
| 13. | 94 | 96 | 90 | 89 | 89 | 0 | 10 | 16 | 10 | 8 | 94 | 86 | 74 | 79 | 82 |
| 29. | 88 | 97 | 93 | 89 | 87 | 0 | 14 | 13 | 8 | 4 | 88 | 83 | 80 | 81 | 83 |
| 30. | 82 | 90 | 85 | 81 | 81 | 0 | 10 | 7 | 4 | 1 | 82 | 80 | 78 | 77 | 80 |
| 32. | 100 | 100 | 102 | 99 | 100 | 0 | 9 | 11 | 9 | 8 | 100 | 99 | 91 | 90 | 92 |
| 36. | 100 | 109 | 106 | 102 | 98 | 0 | 10 | 6 | 4 | 1 | 100 | 99 | 100 | 98 | 97 |
| 40. | 90 | 96 | 92 | 90 | 90 | 0 | 15 | 11 | 3 | 1 | 90 | 81 | 81 | 87 | 89 |
| 41. | 88 | 93 | 90 | 89 | 88 | 0 | 8 | 10 | 10 | 2 | 88 | 85 | 80 | 79 | 86 |
| 47. | 92 | 101 | 95 | - | 91 | 0 | 15 | 14 | - | 7 | 92 | 86 | 81 | - | 84 |
| 50. | 85 | 89 | 86 | 84 | 84 | 0 | 6 | 13 | 6 | 4 | 85 | 83 | 73 | 78 | 80 |
| 64. | 93 | 98 | 101 | 93 | 90 | 0 | 4 | 7 | 8 | 4 | 93 | 94 | 94 | 85 | 86 |
| 77. | 90 | 101 | 94 | - | 87 | 0 | 5 | 5 | - | 4 | 90 | 96 | 89 | - | 83 |
| 84. | 90 | 104 | 96 | - | 85 | 0 | 11 | 14 | - | 5 | 90 | 93 | 82 | - | 83 |
| 100. | 88 | 98 | 92 | - | 89 | 0 | 13 | 9 | - | 2 | 88 | 85 | 83 | - | 87 |

not been included in this series.

A typical graphic response is indicated in graph 5.

(1) The curve for total sugar shows how slight is the hyperglycaemia following fructose ingestion. In only 3 cases out of 20 does the maximum rise exceed 10 mgms. per 100 c.c. and the highest maximum value obtained was 14 mgms. per 100 c.c. (Table III).

(2) The maximum values both for total sugar and for fructose were found in the majority of cases during the first hour after ingestion. Of the two cases which fall outside this range, one, No. 17, should in all probability be considered within it, since there is only 1 mgms. per 100 c.c. difference between the 1 hour and 1½ hours values. More light will be thrown upon the second case as the numbers of normals investigated increases. The results so far as total sugar is concerned agree with those of Tallermann (1923) and of Joliffe (1929) although we find a wider normal range than was allowed for by Spence and Brett (1921).
It should be stated that the maximum figures obtained do not necessarily represent the highest values attained since the peak of the curves may well be missed in the half-hourly samples. Application of the method to capillary blood will render information on this point.

(3) In all cases the blood glucose values decrease, the majority showing a tendency to return to normal within /

within 2 hours. This significance of this feature has already been noted.

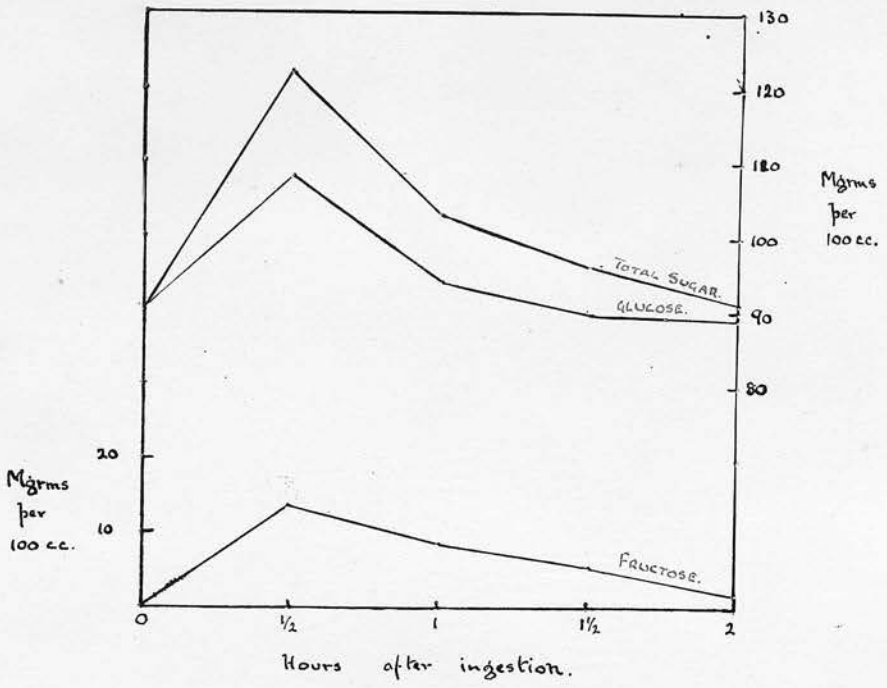
(4) The maximum values for blood fructose in this series lie between 5 and 18 mgms. per 100 c.c. and the mean for the series is 12.8 mgms. per 100 c.c. At the end of two hours values varying from 0 - 8 mgms. per 100 c.c. with an average of 4.0 mgms. per 100 c.c. were found. In the light of what has already been said these figures are difficult of interpretation but we consider that they represent true fructose values.

We may, therefore, define a "normal levulose tolerance" as one which permits a maximum blood levulose rise of not more than 20 mgms. per 100 c.c. to be attained within 1 hour after the ingestion of 50 grams of sugar. At the end of the second hour the curve should show a fall to a value certainly below 10 mgms. per 100 c.c. Coincident with these changes the blood glucose shows a fall below the fasting level and a return towards its initial figure during 2 hours.

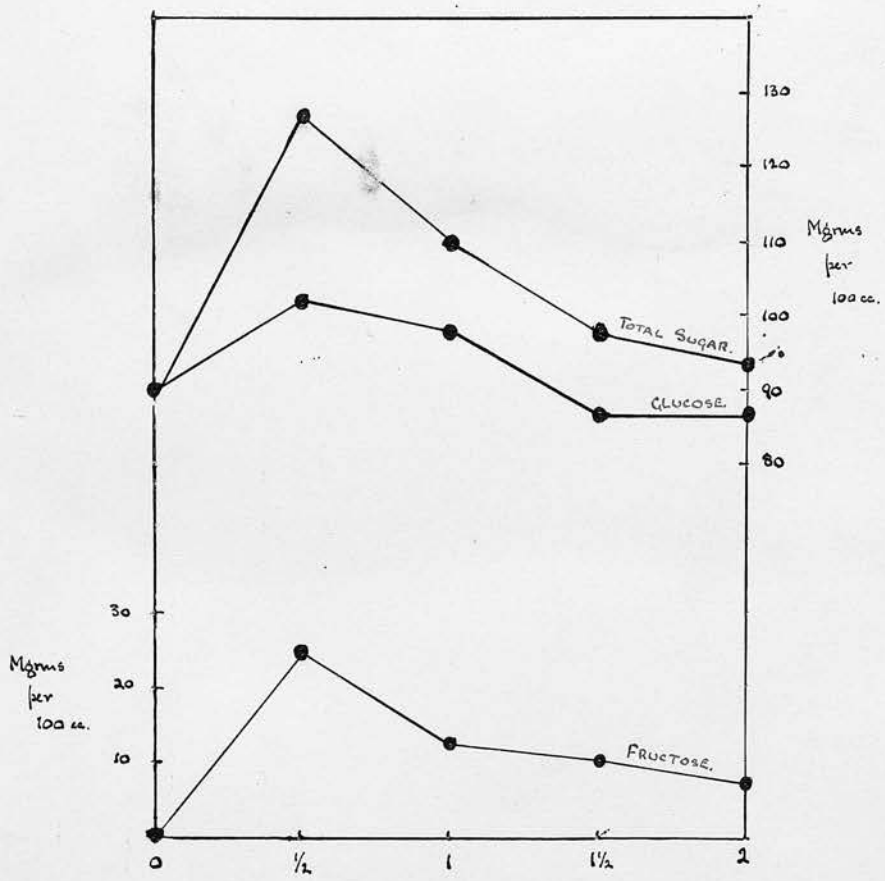
It should be noted that in the old form of carrying out the test a rise of 30 mgms. per 100 c.c. in the total blood sugar was considered to be the highest value allowable for a normal tolerance. That mistakes were frequently made in both directions if this criterion is alone applied is evident from

a /

CASE 9.




CASE 72.



a consideration of cases No. 9 and No. 72.

No. 72. Male, 59. Pain in right hypochondrium and loss of weight for 12 months. Liver palpable about 2" below costal margin: irregular and firm. I.I.36: V.B. +ve biphasic.

This case, if interpreted by the old standard, would be considered to have a normal liver function in respect of levulose: the improved method shows disturbance of function.

No. 9. Male, 24. Anxiety neurosis. If interpreted by the old method would be considered to have some disturbance of liver function. Estimation of blood fructose, however, reveals a normal levulose curve, and shows that the increase in total blood sugar is entirely due to an abnormal rise in blood glucose.

Although these are two examples only, we have several times found this discrepancy to arise in the course of examining over one hundred cases by this method. Error occurs more frequently under the first head, namely a case normal by the old standard is abnormal by the new.

Consideration of the response to the administration of levulose in older people has led us to the view that, just as in the case of glucose, the rapidity with which levulose is removed from the blood is diminished as a result of which higher values for blood levulose are found than those we have described for the age group 20 - 40, and consequently care is necessary before interpreting curves in old people.

Table /

Table 4.

Mgms. per 100 c.c Blood.

| Case. | Age. | Diagnosis. | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 60. | 72. | Urinary infection. | 193. | 264. | 325. | 338. | 286. | 0. | 13. | 13. | 21. | 12. | 193. | 251. | 312. | 317. | 274 |
| 61. | 67 | Urinary infection Arteriosclerosis. | 90. | 138. | 121. | 115. | 110. | 0. | 23. | 16. | 15. | 16. | 90. | 115. | 105. | 100. | 94 |
| 88. | 65. | Arteriosclerosis. | 119. | 130. | 166. | - | 148. | 0. | 10. | 17. | - | 20. | 119. | 120. | 149. | - | 128 |
| 89. | 69. | Angina pectoris. | 88. | 104. | 98. | - | 86. | 0. | 26. | 28. | - | 13. | 88. | 78. | 70. | - | 73 |
| 99. | 60. | Arteriosclerosis. | 75. | 129. | 141. | - | 130. | 0. | 26. | 28. | - | 20. | 75. | 103. | 113. | - | 110 |

Table 4. summarises the results found in a number of cases all over the age of sixty.

Protocols:-

Case 60. Female. Age 72. 7 st. 10. Diabetic 15 yrs. Stabilized on diet:- Cals 1518. CHY 80. Insulin 15-15. Dysuria 3 wks. B.coli infection urinary tract. Radial arts. not thickened. B.P. 126/76. W.R. -ve. Blood urea-N 36 mgms/100 c.c. Urea range test impaired.
Diagnosis:- Diabetes mellitus: Pyelo-cystitis.

Case 61. Female. Aged 67. 7 st. 2. Haematuria and dysuria 8 wks. B.coli in urine. Radial arteries thickened. B.P. 152/94. W.R. -ve. R.b.c.s 2.6. Hb.50 . Blood urea-N 26 mgms/100 c.c. Uroselectan - impaired renal function.
Diagnosis:- Arteriosclerosis: urinary infection.

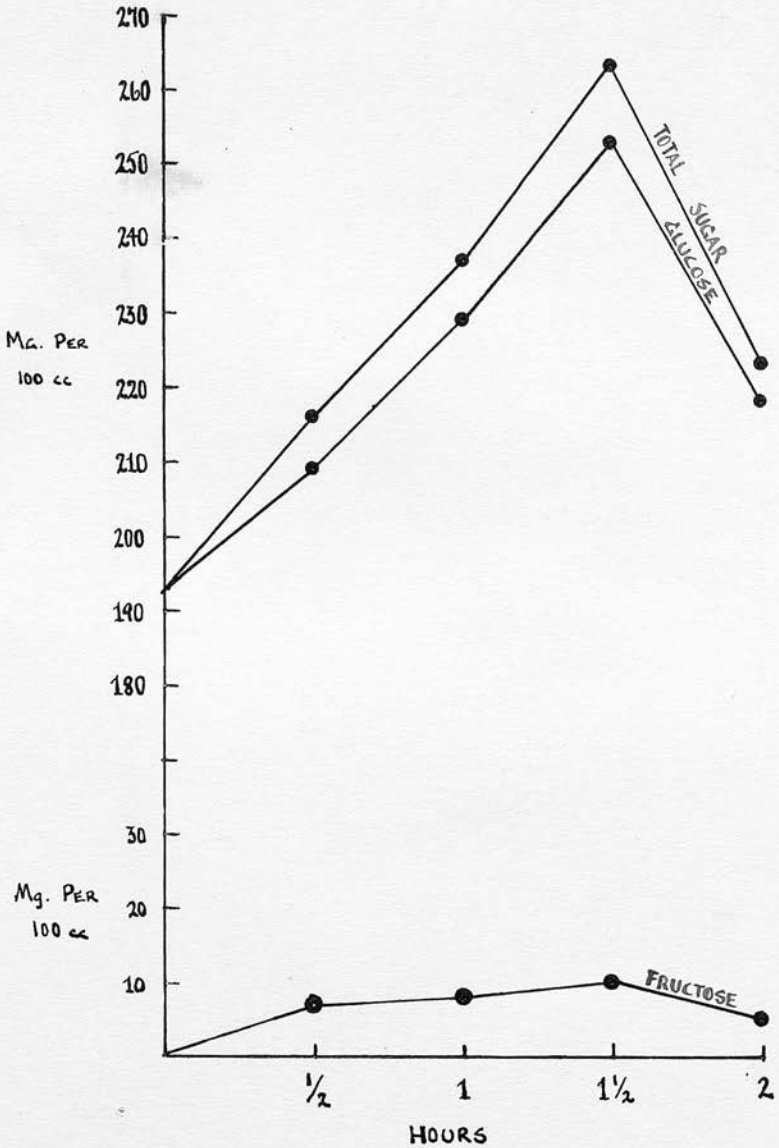
Case 88. Male. Age 65. 8 st. 9. Attacks of severe praecordial pain for 14 days. Dyspnoea on exertion. Flatulent dyspepsia. Radial arteries thickened. B.P. 140/110. W.R. -ve. No glycosuria.
Diagnosis:- Arteriosclerosis: angina pectoris.

Case 89. Male. Age 69. 9 st. 10. Thirst and polyuria 5 months. Aching pain left heel 6 weeks. Stable on cals 1919. CHY 90. Insulin 10-15-5. Radial arteries markedly thickened. B.P. 145/75.
Diagnosis:- Arteriosclerosis: senile diabetes: dry gangrene.

Case 99. Male. Age 60. 10 st. 4. Intermittent claudication 5 yrs. Severe pain in rt. foot 1 week. Radial arteries thickened. B.P. 150/98. W.R. -ve. No glycosuria.
Diagnosis:- Arteriosclerosis: dry gangrene.

Case 60 is a diabetic. Even so, however, the fructose curve would not have shown any abnormality in a younger person. Case 60 had no evidence of arteriosclerosis /

Graph. 6.


270
260
250
240
230
220
210
200
190
180
Mg. Per 100 cc
TOTAL SUGAR
GLUCOSE
30
20
10
Mg. Per 100 cc
FRUCTOSE
½
1
1½
2
HOURS

arteriosclerosis, but cases 88, 89 and 99 all had arterial disease and No. 88 had intermittent glycosuria in addition. The possibility of impaired levulose tolerance in older people is thus established and in one case, No. 88, is associated with high total sugar and glucose curves, suggesting some impairment of pancreatic function. It may be noted that Cases 60 and 61 illustrate the value of the test in another connection, namely prior to the administration of drugs known to be toxic to the liver. These two patients were to have been given acriflavine intravenously but in view of the results obtained with levulose other treatment for urinary infection was substituted.

Diabetes Mellitus and Other Glycosurias.

In uncomplicated diabetes mellitus the levulose tolerance test is, of course, disturbed by reason of the fact that the organism has difficulty in disposing of the excess glucose formed from the ingested levulose and this fact gives rise to one of the major fallacies in the interpretation of the test when total blood sugar only is estimated. We have had an opportunity of investigating several cases of diabetes by the new method and the results are indicated in Table No. 5. a typical curve from a mild diabetic being shown in graph No. 6.

In /

Table 5.

Diabetes Mellitus.

| Subject. | Mgms. per 100 c.c. whole blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 16. | 112 | 148 | 130 | 114 | 111 | 0 | 11 | 10 | 9 | 10 | 112 | 137 | 120 | 105 | 101 |
| 18. | 182 | 259 | 298 | 307 | 324 | 0 | 16.5 | 14 | 8 | 21.5 | 182 | 252.5 | 284 | 299 | 302.5 |
| 24. | 171 | 205 | 186 | 180 | 175 | 0 | 16 | 15 | 16 | 10 | 171 | 189 | 171 | 164 | 165 |
| 34. | 193 | 216 | 237 | 264 | 223 | 0 | 7 | 8 | 10 | 5 | 193 | 209 | 229 | 254 | 218 |
| 60. | 193 | 264 | 325 | 338 | 286 | 0 | 13 | 13 | 21 | 12 | 193 | 251 | 312 | 317 | 274 |
| 43. | 106 | 129 | 183 | - | 246 | 0 | 13 | 15 | - | 13 | 106 | 116 | 168 | - | 233 |
| 55. | 114 | 150 | 149 | 169 | 140 | 0 | 12 | 27 | 32 | 20 | 114 | 138 | 122 | 137 | 120 |

In all these cases the fructose curve is normal whereas the total blood sugar values ascend to high levels and in the more severe cases have by no means returned to their initial figures. This rise is due to blood glucose. Unfortunately we are not able to say whether, in the diabetic subject, the hyperglycaemic response to 50 grams of glucose is greater than that with 50 grams of levulose as it is the normal person. Investigations along these lines might well throw light on the methods of removal of levulose from blood and will be made when opportunity offers.

Protocols:-

Case 16. Female. Age 40. Diabetic for 12 months. Stable on Cals 1956. CHY 101.
Diabetes mellitus - uncomplicated.

Case 18. Male. age 35. Diabetic for 2 yrs. Stable on Cals 2859. CHY 132. Insulin 32-32.
Diabetes mellitus - uncomplicated.

Case 24. Male. Age 53. Diabetic for 6 yrs. Stable on Cals 2833. C170.
Diabetes mellitus - uncomplicated.

Case 43. Female. Aged 73. 11 st. 6. Weakness and loss of weight 1 month. Flatulent dyspepsia, thirst and polyuria. Diet Cals 1518. CHY 78. Insulin 10-10-10. Urine contains sugar. Jaundice 5 wks. I.I. 150 and progressive V.B. immediate direct. Liver ? enlarged. Vague mass in epigastrium with tenderness on deep palpation. No enlargement spleen. No free fluid. Radial arts. not thickened.
B.P. 120/68. W.R. -ve.
Diagnosis (clinical):- Carcinoma of pancreas: diabetes mellitus: obstructive jaundice.

Case 55. Female. Aged 56. 10 st. 4. Diabetic symptoms 6 months. Stable on Cals 1660 CHY 71. Dysuria, haematuria 14 days. B.coli infection. Radial arteries not thickened.
B.P. /

Table 6.

| Case. | Diagnosis. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| | | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 27. | Acromegaly. | 92 | 101 | 97 | 95 | 93 | 0 | 8 | 11 | 2 | 4 | 92 | 93 | 86 | 93 | 89 |
| 31. | Toxic goitre. | 106 | 135 | 112 | 108 | 104 | 0 | 10 | 7 | 4 | 3 | 106 | 125 | 105 | 104 | 101 |
| | before optn. | 290 | 340 | 429 | 387 | 322 | 0 | 54 | 116 | 80 | 75 | 290 | 286 | 313 | 307 | 247 |
| | Toxic goitre after optn. | 129 | 165 | 160 | - | 151 | 0 | 20 | 11 | - | 9 | 129 | 145 | 149 | - | 142 |

B.P. 136/76. W.R. -ve. 15 years history of excessive alcohol consumption.
Diagnosis:- Diabetes mellitus: B. coli cystitis.

Case 34. Male. Aged 62. 9 st. 10. Diabetic sympts. 3 weeks. Stable on Cals 2100. CHY 85. Insulin 15-10. Radial arts. thickened and calcified. B.P. 230/115. W.R. -ve. Diabetes mellitus: arteriosclerosis.

Cases 43 and 55 are complicated - by obstructive jaundice and by urinary infection respectively. Particular attention should be directed to case 55 in which there was a prolonged history of alcoholism although no clinical evidence of hepatic disease, the levulose test shows disturbance of hepatic function. Table 6. shows the results obtained in three cases of endocrinal dysfunction. No.s 22 and 49 are from the same case before and after thyroidectomy. The extreme values in the fructose values before operation will be noted, and, although no conclusions can, of course, be drawn from a single case, this result is interesting in view of the work of Cameron and Karunaratne (1936) and Youmans and Warfield (1935) on degenerative changes in the liver in thyreotoxicosis. It occurs to us, however, that in the presence of blood sugar values of the order of 400 mgms. per 100 c.c., the diphenylamine method for levulose may not be accurate. Further work requires to be done on this point and also on the question of liver function in thyreoid disease.

Protocols:-

Table 7.

| Case. | Diagnosis. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| | | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 26. | Obstructive jaundice. Gall-stones. | 100 | 128 | 119 | - | 109 | 0 | 3 | 6 | - | 3 | 100 | 125 | 113 | - | 106 |
| 28. | Arsenical jaundice. | 99 | 111 | 118 | 120 | 105 | 0 | 7 | 11 | 10 | 9 | 99 | 104 | 107 | 110 | 96 |
| 35. | Carcinoma of stomach. Obst. jaundice. Metastases in Liver. | 120 | 140 | 142 | 139 | 150 | 0 | 12 | 12 | 10 | 4 | 120 | 128 | 130 | 129 | 136 |
| 59. | Arteriosclerosis. | 83 | 90 | 101 | 92 | 84 | 0 | 7 | 10 | 8 | 4 | 83 | 83 | 91 | 84 | 81 |
| 63. | Empyema Pyelocystitis. | 86 | 100 | 92 | 87 | 84 | 0 | 5 | 5 | 4 | 3 | 86 | 95 | 87 | 83 | 81 |
| 67. | Supra-renal tumour. | 82 | 110 | 95 | - | 84 | 0 | 6 | 3 | - | 2 | 82 | 104 | 92 | - | 82 |
| do. | do. | 90 | 128 | 105 | - | 93 | 0 | 4 | 5 | - | 2 | 90 | 124 | 100 | - | 91 |
| do. | do. | 90 | 101 | 94 | - | 87 | 0 | 5 | 5 | - | 4 | 90 | 96 | 89 | - | 83 |
| 73. | Tuberculous peritonitis. | 93 | 105 | 121 | - | 98 | 0 | 6 | 12 | - | 10 | 93 | 99 | 109 | - | 88 |
| 86. | Obstr. jaundice. Gall-stones. | 85 | 114 | 108 | - | 102 | 0 | 12 | 16 | - | 11 | 85 | 102 | 92 | - | 91 |

Protocols:-

Case 27. Female. Aged 27. 10 st. 7. Symptoms of 1 year duration. No glycosuria. B.M.R. + 6%
Diagnosis:- (Clin.X.Ray). Chromophil Adenoma of pituitary: acromegaly.

Case 31. Female. Age 39. 8 st. 13. Dyspnoea on exertion: palpitations: perspiration 16 mns. No glycosuria. B.M.R. + 20%. Rad. Arts. not thickened. B.P. 150/72. W.R. -ve.
Diagnosis:- Toxic goitre.

Case 49. Female. Age 27. 6 st. 10. Diabetic symptoms 3 yrs. Now more or less stable on Cals CHY. Insulin 17-17. Nervousness, perspiration, loss of weight and tremor 2 mns. Thyroid enlargement 14 days. P.R. 130 per min. B.P. 150/40. B.M.R. + 48%.
Diagnosis:- Diabetes: hyperthyroidism. Second test performed 3 weeks after sub-total thyroidectomy. Wt. 7 st. 1. P.R. 90 per min. No toxic symptoms. B.M.R. + 2%.

During our investigations of the value of the levulose tolerance test as an aid to diagnosis we have encountered several cases in which the only abnormality in the results is that the glucose curve rises instead of falls. This finding has occurred in a miscellaneous collection of material and we are unable at present to interpret the results which are, however, set forth in Table 7.

Table 8.

Cardiac Failure.

| Case. | Age. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| | | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 | 0 | ½ | 1 | 1½ | 2 |
| 14. | 66 | 114 | 139 | 129 | 119 | 117 | 0 | 20.5 | 14 | 12 | 13 | 114 | 118.5 | 115 | 107 | 104 |
| 19. | 16 | 104 | 122 | 109 | 105 | 102 | 0 | 6.5 | 19 | 10 | 4 | 104 | 115.5 | 90 | 95 | 88 |
| 20. | 41 | 129 | 136 | 130 | 128 | 128 | 0 | 6 | 13 | 8 | 8 | 129 | 130 | 117 | 120 | 120 |

From what has beensaid in the previous part of this essay it is in disease of the liver that the most marked disturbances from the normal might be expected. The following pages are devoted to a consideration of the levulose tolerance test in subjects having, or suspected of having, hepatic dysfunction.

Table 8. indicates the response obtained in 3 cases of cardiac failure all with enlargement of the liver.

Protocols:-

Case 14. Male. Age 66. Severe exertion dyspnoea, 1½ years. Cough, flatulent dyspepsia 8 mns. Orthopnoea, cyanosis, considerable oedema, and bilateral hydrothorax. No ascites nor jaundice.
Liver 2" enlarged, regular, firm and tender.
Radial arteries markedly thickened. B.P.164/90.
Diagnosis:- Arteriosclerosis. Hypertensive cardiac failure.

Case 19. Male. Age 16. Dyspnoea on exertion with cyanosis and oedema 1 yr. No ascites nor jaundice.
Radial arteries not thickened. B.P. 126/80.
Liver 4" enlarged, smooth, regular and slightly tender.
Diagnosis:- Mitral stenosis and incompetence (rheumatic); congestive cardiac failure.

Case 20. Female. Age 41. Exertion dyspnoea 6 mns. Patient very cyanosed, severely orthopnoeic and drowsy.
Moderate oedema and slight ascites. No Jaundice.
Liver 5" enlarged, smooth, regular and tender.
Diagnosis:- Mitral stenosis and incompetence: congestive cardiac failure.

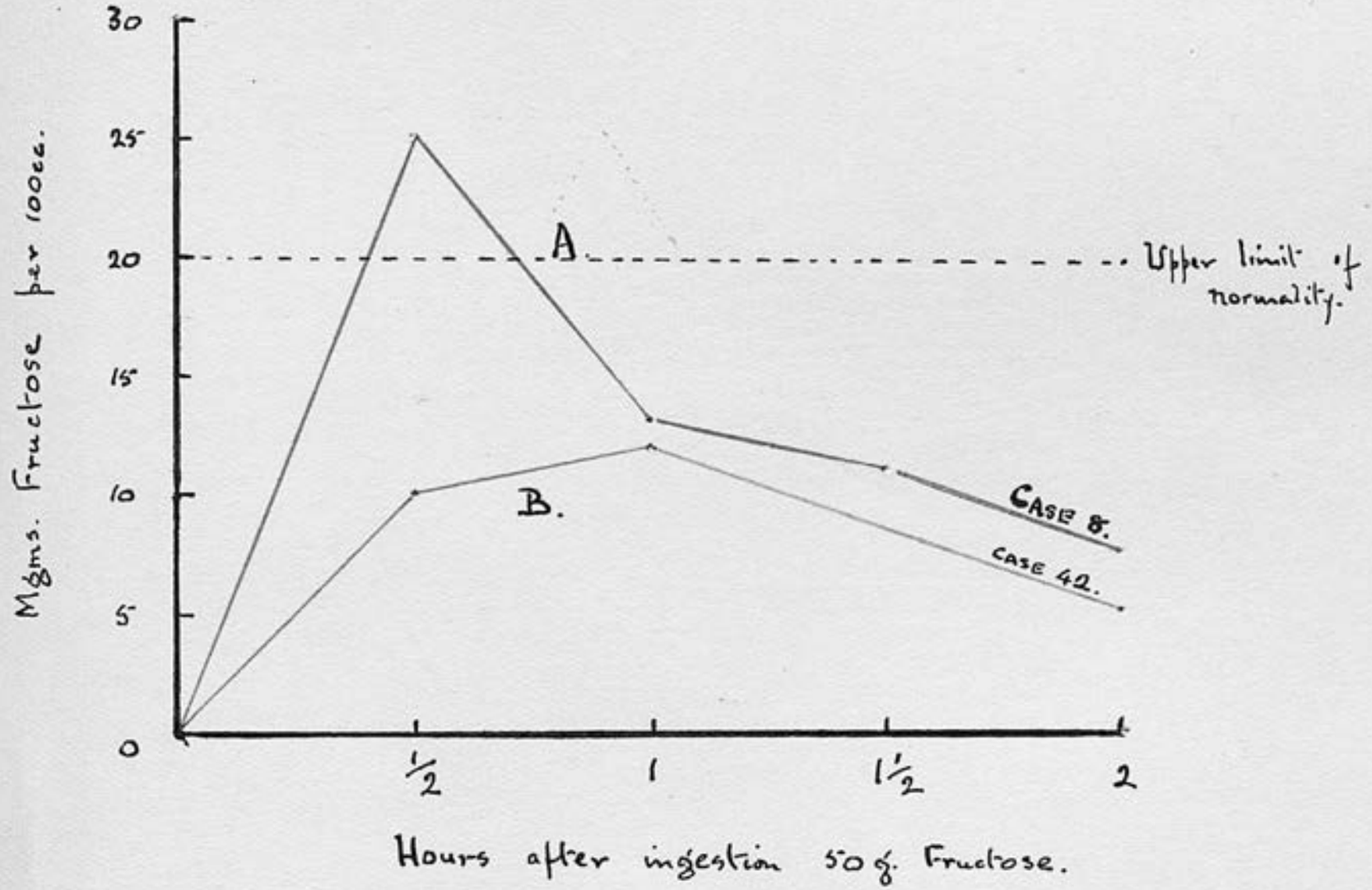
These cases illustrate the fact that there may be marked enlargement of the liver without disturbance of the levulose test. All three patients were congestive /

Table 9.

Toxic Jaundice.

| No. | Diagnosis. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| 5. | during Arsenical jaundice | 100 | 116 | 129 | 118 | 104 | 0 | 10 | 24 | 17.5 | 10 | 100 | 106 | 105 | 100.5 | 94 |
| | after | 86 | 98 | 91 | 89 | 88 | 0 | 18 | 17 | 12 | 8 | 86 | 80 | 74 | 77 | 80 |
| 95. | Septicaemia. Toxic jaundice. | 99 | 129 | 139 | - | 130 | 0 | 28 | 34 | - | 27 | 99 | 101 | 105 | - | 103 |

Graph. 7.


Mgms. Fructose per 100cc.
30
25
20
15
10
5
0
A
Upper limit of normality.
B.
CASE 8.
CASE 42.
½
1
1½
2
Hours after ingestion 50 g. Fructose.

congestive but in none was there jaundice, suggesting the possibility that disturbance of the levulose tolerance test may not occur until the excretory mechanism of the liver is impaired. The suggestion has, in fact, been made that the levulose test shows the most marked disturbance in those patients who are jaundiced. That impairment of levulose tolerance is not necessarily associated with jaundice is indicated in graph 7 , which shows the results obtained so far as levulose alone is concerned, on two subjects only one of which was jaundiced.

Curve A. is from á case of lymphadenoma in which the liver was enlarged but showing no jaundice, while curve B. was obtained in a case of obstructive jaundice due to carcinoma of the common bile duct in which the icteric index was 113, there being no palpable enlargement of the liver.

Toxic Jaundice. Two cases of toxic jaundice have been examined, the results being indicated in Table 9 .

Case 5 is of interest in that it demonstrated the greater delicacy of the direct levulose estimation when trying to assess liver damage. The first curve was obtained during the height of the jaundice (Icteric index 70: liver 2" enlarged and tender) and if assessed on total blood sugar figures alone - the old method of interpretation - would be considered only as /

Table 10.

Catarrhal Jaundice.

| No. | Icteric Index. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| 17. | 30. | 100 | 130 | 146 | 146 | 139 | 0 | 26 | 22 | 18 | 25 | 100 | 104 | 124 | 128 | 114 |
| 33. | 164. | 105 | 109 | 119 | 110 | 107 | 0 | 12 | 18 | 9 | 6 | 105 | 97 | 101 | 101 | 101 |
| 62. | 24. | 80 | 105 | 111 | 96 | 84 | 0 | 15 | 9 | 6 | 4 | 80 | 90 | 102 | 90 | 80 |
| 75. | 31. | 92 | 161 | 130 | - | 99 | 0 | 13 | 13 | - | 11 | 92 | 148 | 117 | - | 88 |
| 78. | 40. | 95 | 116 | 124 | - | 131 | 0 | 30 | 37 | - | 15 | 95 | 86 | 87 | - | 116 |
| 79. | 36. | 82 | 107 | 145 | - | 138 | 0 | 14 | 20 | - | 14 | 82 | 83 | 125 | - | 124 |

as a border-line case. Direct estimation of levulose, however, demonstrates definite liver damage. A month later the same patient gave the second curve (Icteric index now 26: liver just palpable below costal margin) and in this both sets of figures fall within normal limits. There is no doubt that the levulose curves demonstrate more clearly than the total blood sugar values both the initial presence of liver dysfunction and its return to the normal state.

Case 95 was a patient aged 71 with empyema and pyonephrosis who later developed septicaemia and toxic jaundice. Curve B. was taken before the development of jaundice and curve A. after (I.I. 36) illustrating impairment of levulose tolerance this time associated with increasing jaundice.

In acute hepatitis one might expect a marked disturbance of liver function. Table 1a shows the results obtained in a series of six cases of which the following are brief clinical notes:-

Protocols:-

Case 17. Male. Age 60. 7 st. 8. Headache, pains in back, jaundice 5 weeks. Liver just palpable and tender. I.I. 30 (one week previously 103) V.B. biphasic. W.R. -ve.

Case 33. Male. Age 31. 8 st. 12. Anorexia and flatulence 3 wks. Jaundice 14 days. Liver 2½" enlarged, firm, regular and not tender. I.I. 164. V.B. biphasic. W.R. -ve.

Case 62. Abdominal pain 3 mns. Severe epigastric pain, vomiting, jaundice 7 days. Liver 1" enlarged, firm and very tender. I.I. 24. V.B. biphasic. W.R. -ve.

Case 75. Female. Age 16. 5 st. 2. Pain rt. side

Table 11.

Obstructive jaundice.

| No. | Icteric Index. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| 42. | 113. | 98 | 123 | 140 | - | 110 | 0 | 10 | 12 | - | 5 | 98 | 113 | 128 | - | 105 |
| 46. | ( 162. | 98 | 119 | 128 | 140 | 127 | 0 | 26 | 34 | 47 | 38 | 98 | 93 | 94 | 93 | 89 |
| | ( 149. | 70 | 134 | 120 | - | 78 | 0 | 14 | 18 | - | 16 | 70 | 120 | 102 | - | 62 |
| 52. | 28. | 86 | 99 | 123 | 109 | 94 | 0 | 19 | 23 | 30 | 15 | 86 | 80 | 100 | 79 | 79 |
| 69. | 120. | 80 | 124 | 103 | - | 93 | 0 | 26 | 26 | - | 16 | 80 | 98 | 77 | - | 77 |
| 74. | 110. | 90 | 156 | 142 | - | 122 | 0 | 12 | 37 | - | 13 | 90 | 144 | 105 | - | 109 |
| 81. | 110. | 108 | 120 | 110 | 107 | 107 | 0 | 20 | 20 | 52 | 47 | 108 | 100 | 90 | 55 | 60 |
| 85. | 57. | 210 | 255 | 231 | - | 289 | 0 | 36 | 38 | - | 16 | 210 | 219 | 283 | - | 273 |
| 87. | 134. | 111 | 139 | 159 | 147 | 140 | 0 | 35 | 34 | 32 | 27 | 111 | 104 | 125 | 115 | 113 |
| 90. | 43. | 112 | 138 | 166 | - | 132 | 0 | 13 | 21 | - | 18 | 112 | 115 | 145 | - | 114 |

side abdomen and sickness 3 wks. Jaundice 18 days. Liver not enlarged but tender on deep palpation. I.I. 31. V.B. biphasic. W.R. -ve.

Case 78. Male. Age 24. 9 st. 2. Upper abdominal pain 14 days. Jaundice 5 days. Liver not enlarged. No tenderness. I.I. 40. V.B. biphasic. W.R.-ve.

Case 79. Female. Age 30. 8 st. 12. Jaundice and lack of energy 1 wk. Liver ½" enlarged and tender. I.I. 36. V.B. biphasic. W.R. -ve.

It will be observed that only two cases - 17 and 78 - are abnormal, while one - 79 - is a border-line case. In only one - 78 - is the disturbance of the levulose curve marked and there is no relationship between the sugar values and the degree of jaundice as measured by the icteric index. Case 33 with an icteric index of 164 actually shows a fall in blood glucose. In view of these results far more clinical material must be collected before more is said on the levulose tolerance test in catarrhal jaundice.

In obstructive jaundice due to a variety of causes, various degrees of disturbance have been found. Table 11. indicates the results obtained and protocols are given below:-

Case 42. Male. age 62. 9 st. 3. Increasing jaundice and loss of weight 2 mns. Liver ? enlarged: tumour in rt. hypochondrium projecting from below costal margin. I.I. 113. V.B. immediate direct. W.R. -ve.
Radial arts. not thickened. B.P. 98/64.
Diagnosis:- Primary carcinoma of common bile duct: obstructive jaundice, (Optn.)

Case 46. Female. Age 51. Jaundice, indigestion, loss of wt. 5 wks. Liver not enlarged but tender on deep pressure. No palpable mass. I.I. 162. V.B. biphasic. W.R. -ve.

12 days after laparotomy I.I. 149. second curve obtained.
Diagnosis:- Adeno-carcinoma of pancreas: obstructive jaundice, (operation).

Case 52. Female. Age 61. Constipation, flatulent dyspepsia and jaundice 2 mns. Liver enlarged and tender. I.I. 28. V.B. delayed direct. W.R. -ve.
Diagnosis:- Stone in common bile duct: obstructive jaundice. (clinical).

Case 69. Male. Age 55. 9 st. 7. Attacks upper abdominal pain for many years. Liver 1" enlarged. I.I. 120. V.B. biphasic. W.R.-ve. Radial arts. not thickened. B.P. 130/90.
Diagnosis:- Cholecystitis and cholangitis: gall stones: obstructive jaundice. (Operation).

Case 74. Male. Age 38. 10 st. 4. Pain in abdomen and diarrhoea, loss of wt. and jaundice 5 wks. Liver 2" enlarged. I.I. 138. V.B. immediate direct.
Diagnosis:- Carcinoma hepatic duct: obstructive jaundice. (operation).

Case 81. Male. Age 79. Constipation with attacks of diarrhoea: lost of wt. and vomiting 6 wks. Jaundice 3 wks. Liver 1" enlarged. I.I. 110. V.B. biphasic.
Diagnosis:- Carcinoma of biliary passages: obstructive jaundice.

Case 85. Female. Age 61. 8 st. 7. Diabetic symptoms 3 yrs. Jaundice, pain in rt. hypochondrium, and vomiting 9 days. Liver 2" enlarged. Palpable gall-bladder. Palpable mass in epigastrium. I.I. 57. W.R.-ve. Radial arts. thickened. B.P. 124/68.
Diagnosis:- Cholecystitis and gall stones: diabetes mellitus: obstructive jaundice: ? carcinoma of pancreas. (clinical: radiological).

Case 87. Male. Age 80. Jaundice, flatulent dyspepsia, loss of wt. 14 days. Liver not felt. I.I. 134. V.B. biphasic. W.R.-ve. Radial arts. thickened. B.P. 130/75.
Diagnosis:- Carcinoma of head of pancreas: obstructive jaundice. (clinical).

Case /

Case 90. Female. Age 52. 11 st. 12. Jaundice 5 wks. Nausea, epigastric pain, and flatulence 3 wks. Liver ½" enlarged, very firm and slightly tender. I.I. 43. W.R.-ve. Radial arts. not thickened. B.P. 126/74.
Diagnosis:- Carcinoma of pancreas: gall-stone: obstructive jaundice. (operation).

This series of cases further confirms the view that there is no relationship between the disturbance of the levulose tolerance test and the depth of jaundice. Every case shows figures abnormal in some respect. The levulose figures exceed the normal in all cases except 42 and 46b., and are suspicious in 90. These three cases, however, present an interesting feature not so far noted, - although the levulose figures may be considered normal, the total sugar curve rises to values in excess of normal, indicating an impairment in glycogenesis on the part of the liver or an insufficient insulin production by the pancreas. It may be noted that in 46 and 90 the pancreas was the seat of a malignant process, whereas in 42 the diagnosis was carcinoma of the common bile duct. Case 46 also reveals an improvement in hepatic function after laparotomy associated with a slight decrease in the depth of the jaundice, whereas the pancreatic insufficiency remains.

Cirrhosis of the Liver. Table 12 summarises the findings in ten cases of cirrhosis of the liver. In 21 and 45 there was no history of alcoholism but both were /

Table 12.

Cirrhosis.

| No. | Mgms. per 100 c.c. blood. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
| 37. | 96 | 105 | 96 | - | 88 | 0 | 18 | 23 | - | 15 | 96 | 87 | 73 | - | 7 |
| 48. | 93 | 101 | 96 | - | 92 | 0 | 15 | 13 | - | 8 | 93 | 86 | 83 | - | 84 |
| 54. | 92 | 100 | 108 | 99 | 93 | 0 | 15 | 22 | 22 | 25 | 92 | 85 | 86 | 77 | 68 |
| 56. | 86 | 99 | 117 | 102 | 92 | 0 | 16 | 11 | 9 | 16 | 86 | 83 | 106 | 93 | 76 |
| 65. | 90 | 139 | 120 | - | 109 | 0 | 9 | 13 | - | 14 | 90 | 130 | 107 | - | 95 |
| 66. | 80 | 127 | 115 | - | 99 | 0 | 11 | 8 | - | 3 | 80 | 116 | 107 | - | 96 |
| 68. | 82 | 114 | 131 | 114 | 84 | 0 | 16 | 29 | 22 | 11 | 82 | 98 | 102 | 92 | 73 |
| 70. | ( 79 | 93 | 121 | - | 135 | 0 | 9 | 13 | - | 25 | 79 | 84 | 108 | - | 110 |
| | ( 94 | 142 | 135 | - | 114 | 0 | 25 | 32 | - | 12 | 94 | 117 | 103 | - | 102 |
| 21. | 97 | 120 | 140 | 176 | 145 | 0 | 19 | 54 | 37 | 35 | 97 | 107 | 86 | 139 | 110 |
| 45. | 84 | 112 | 106 | 93 | 85 | 0 | 8 | 24 | 28 | 32 | 84 | 104 | 82 | 65 | 53 |

were diagnosed clinically as cirrhosis, the diagnosis in the case of 21 being confirmed at autopsy. Both subjects were jaundiced (icteric indices 108 and 20 respectively) and both show an abnormal response. Cases 65 and 66 both gave a history of prolonged excessive consumption of alcohol and both had haematemesis on admission to hospital. Neither case was jaundiced, nor was the liver enlarged in either. The fructose curve is normal in both but again we find an excessive rise in the total blood sugar values due to the fact that the blood glucose is abnormally increased. Since there is no reason from a clinical point of view to suppose that there is any pancreatic defect, and since in neither was the symptomatology nor the radiological findings at all suggestive of any other lesion which might have shown itself by haematemesis, are we to suppose that an increase in the total sugar due to an impairment of glycogenesis is an early sign of cirrhosis of the liver? As the lesion progresses this might then be followed by a return to normal of the total sugar values as the levulose curve becomes abnormally raised, the fall in total sugar being occasioned by the fact that the glucose is now delivered more slowly as the liver's power of converting levulose into glucose becomes impaired. This, of course, is speculation: it can only /

Table 13.

Carcinoma of Liver.

Mgms. per 100 c.c. blood.

| No. | Total Sugar. | | | | | Levulose. | | | | | Glucose. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 90 | 102 | 122 | 99 | 90 | 0 | 32.5 | 37.5 | 16 | 2.5 | 90 | 69.5 | 84.5 | 83 | 87.5 |
| 51. | 81 | 86 | 80 | - | 79 | 0 | 6 | 10 | - | 5 | 81 | 80 | 70 | - | 74 |
| 72. | 88 | 108 | 99 | - | 89 | 0 | 27 | 11 | - | 4 | 88 | 81 | 88 | - | 85 |
| 76. | 104 | 153 | 120 | - | 99 | 0 | 35 | 28 | - | 5 | 104 | 118 | 92 | - | 94 |
| 80. | 140 | 210 | 180 | 160 | 149 | 0 | 18 | 32 | 32 | 25 | 140 | 192 | 148 | 128 | 124 |
| 82. | 88 | 102 | 119 | 110 | 101 | 0 | 16 | 31 | 25 | 18 | 88 | 86 | 88 | 85 | 83 |
| 83. | 78 | 94 | 107 | - | 116 | 0 | 22 | 25 | - | 21 | 78 | 72 | 82 | - | 95 |
| 90. | 84 | 116 | 110 | - | 88 | 0 | 12 | 10 | - | 6 | 84 | 104 | 90 | - | 82 |

Table 14.

Hepatic Syphilis.

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38. | 110 | 130 | 141 | 133 | 115 | 0 | 22 | 23 | 12 | 9 | 110 | 108 | 118 | 121 | 106 |
| 91. | 101 | 108 | 117 | - | 125 | 0 | 17 | 18 | - | 10 | 101 | 91 | 99 | - | 115 |

only become more than that by following closely these, and other, similar cases. Cases 48 and 56 diagnosed clinically as alcoholic cirrhosis give normal responses, although the total sugar values of 56 attain a suspiciously high maximum. Case 70 was clearly a case of alcoholic cirrhosis: the higher figures in the second test, performed a month after the first, coincide with a definite deterioration in clinical condition.

Carcinoma of the Liver. Table 13.

Protocols:-

Case 1. Male. Age 40. 7 st. 7. Flatulence, heart-burn, and abdominal discomfort for several years. Jaundice 9 mns. Loss of wt. Liver 4" enlarged: gall-bladder palpable. I.I. 100 W.R. -ve.
Diagnosis:- Carcinoma of pancreas: metastases in liver: obstructive jaundice. (Operation, autopsy).

Case 51. Male. Age 60. 8 st. 12. Oedema of feet and ankles; ascites 4 wks. Liver 4" enlarged. Surface nodular, border irregular, firm and not tender. No jaundice. W.R. +++.
Radial arteries calcified. B.P. 220/60.
Diagnosis:- Cardiovascular syphilis: Carcinoma of liver (No primary focus found ).

Case 72. Male. Age 49. 8 st. 5. Pain in upper abdomen, loss of wt. 6 mns. Increasing jaundice 14 days. Liver - mass projecting below c.m. no general enlargement. I.I. 36. W.R.-ve.
Diagnosis:- Carcinoma of liver (no primary focus found).

Case 76. Male. Age 59. 8 st. 5. Pain in rt. hypochondrium, loss of wt. 12 mns. Liver - palpable mass projecting 2" below c.m., liver not generally enlarged. I.I. 67. W.R.-ve.
Diagnosis:- Carcinoma of liver (no primary focus found).

Case/

Case 80. Male. Age 66. Pain in epigastrium with increasing jaundice and progressive loss of wt. 6 mns. Liver within ½" of umbilicus, nodular surface and irregular edge, very firm, and tender. I.I. 178. W.R.-ve. Radial arts. not thickened.
Diagnosis:- Carcinoma of liver (no primary focus found).

Case 82. Male. Age 63. Constipation, abdominal pain, passage of blood, 14 days. Liver not enlarged. No jaundice. W.R.-ve. Radial arteries markedly thickened. B.P. 195/110.
Diagnosis:- Carcinoma pelvic colon: arteriosclerosis. No metastases found in liver at operation.

Case 83. Female. Age 28. 7 st. 9. Abdominal pain and swelling 2 mns. Liver 2" enlarged, hard, irregular and tender. I.I. 16. W.R.-ve.
Diagnosis:- Primary carcinoma of liver. (Autopsy).

Case 90. Male. Age 57. Increasing jaundice 6 wks. Abdominal swelling, loss of wt. 1 month. Liver 1½" enlarged, firm, smooth and not tender. I.I. 36. W.R.-ve.
Diagnosis:- Primary carcinoma of liver. (Autopsy).

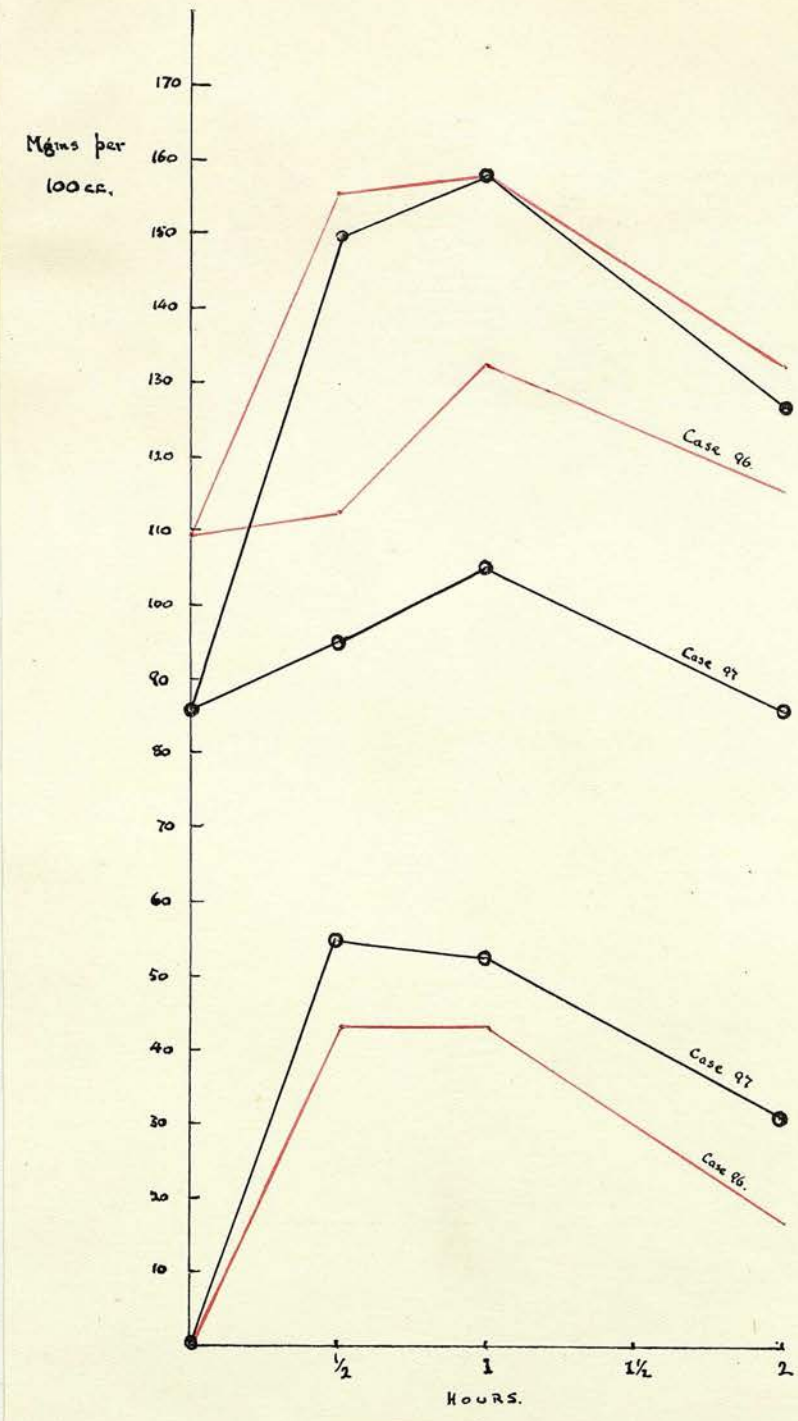
Of these eight cases only two show normal levulose values - 51 and 90 - and of these two 90 is abnormal if interpreted on basis of total sugar figures, and the diagnosis in the case of 51 is doubtful. Case 82 is a border-line case with regard to total sugar but definitely abnormal in respect of the levulose curve. Contrasting with this is 72 which gives abnormal levulose figures but is normal if interpreted on the basis of total sugar. In all cases except 80 the blood glucose shows a fall below the fasting level at some time during the two hour period and one must bear /

bear in mind that had specimens been taken at more frequent intervals more pronounced falls might have been found. Particular attention should be directed towards Case 80. The clinical diagnosis was carcinoma of the liver, either primary or secondary, but no primary focus was found in spite of complete radiological investigations of the alimentary tract and intensive clinical examination. Our investigations make this case one of carcinoma of the pancreas - high fasting blood sugar with a subsequent rise in the total blood sugar values well above the maximum allowable and a marked rise in the blood glucose. We conclude also that the liver is the seat of metastases since the fructose curve itself shows values in excess of normal.

Hepatic syphilis. Table 14. One cannot draw any conclusion as to the disturbance of the levulose tolerance test in syphilitic cirrhosis of the liver from a consideration of two cases, suffice it to say that the abnormal values in Case 38 were associated with a much less favourable clinical condition, the patient having marked ascites and oedema of the legs, a secondary anaemia, an icteric index of 85 and considerable hepatic enlargement.

Hepato-lienal fibrosis. Graph 8. shows the results /

Graph 8.


Mgms per 100 cc.
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Case 96
Case 97
Case 97
Case 96.
½
1
1½
2
HOURS.

results obtained in two such cases. Case 96 was a female aged 58, who had had symptoms for 15 years while Case 97 was a female aged 17 who had been perfectly well until six months before the test was performed. The very close similarity between the curves obtained will be noted, while the very high values attained by the levulose figures attract attention.

In conclusion it might be well to devote some consideration to the value of the levulose tolerance test in the improved form suggested by us in order to determine whether or not it can be of value in the diagnosis of hepatic disorder. Objections to this and other laboratory tests devised for the same purpose have been raised frequently in the past, but the majority of these are based on the experiments of Mann (1925) who was able to remove up to four-fifths of the liver in dogs before metabolic disturbances became evident. In this connection, however, it is well to bear in mind, as Kimball (1932) pointed out, "the vast difference between one-fifth of a healthy liver and an entire liver, all of which is diseased, however mild that disease may be." That it is, in fact /

fact, possible to detect small degrees of liver damage by biochemical tests has been shown by Rosenthal (1922) who was able to detect the removal of as little as 12% of the liver of rabbits by measuring the rate of disappearance of phenoltetrachlorphthalein from the blood. It is important to remember, however, in dealing with an organ such as the liver, which possesses a large number of unrelated functions, that a disturbance of one function, even though it be marked, does not necessarily mean a disturbance of all. That this is, in point of fact, true, is indicated by the lack of relationship between our results with the levulose tolerance test and the depth of jaundice present at the time. The present investigation has undoubtedly shown that the improved form of the levulose tolerance is a marked improvement on the older method of performing the test, in the first place because a number of interfering factors are eliminated, a more exact investigation of hepatic function, therefore, being rendered possible, and secondly because a wider field of investigation is thus introduced, it being possible not only to gain information as to the efficiency of the reaction fructose → glucose, which, as we have shown, depends mainly on the liver, but also to investigate /

investigate the reaction glucose → glycogen which depends to a large extent on the integrity of pancreatic function, although the process itself occurs in the liver. Such knowledge as has been shown may be of extreme significance in the investigation of a case having both hepatic and pancreatic disturbance, and in the diagnosis of obscure and difficult cases in which, possibly, other methods of examination have failed to lead to an exact diagnosis. This being so, and since some subjects deviate from the strictly normal in showing only a rise in the glucose curve instead of a fall - a fact which is not, at the moment, susceptible of exact interpretation - we consider that simultaneous estimations of 'total blood sugar' glucose, and levulose are essential parts of the test.

From this investigation of over one hundred cases by this procedure we find that in liver disease the blood levulose may reach a maximum, half to one hour after ingestion, of over 20 mgms. per 100 c.c., and that it does not fall to the same extent as in normal persons at the end of two hours. The same case does not necessarily show both these abnormalities and in four cases the maximum levulose value has been found at the end of two hours. For this reason we lay greater stress on the attainment of a high maximum as an indication of liver dysfunction, but the factors responsible /

responsible for a high levulose figure at the end of the test are under consideration. In this connection one remembers the vexed question of the renal threshold for fructose which is possibly a mediating factor in this respect.

Many interesting problems have arisen in the course of this investigation and further work on the application of the levulose tolerance test to cases having, or suspected of having, hepatic dysfunction is in progress. In addition it is hoped to investigate more thoroughly certain aspects of levulose metabolism along the following lines which have suggested themselves to us during our review of what is known of the metabolism of levulose in the first part of this essay.

1. The metabolism of fructose in the depancreatised animal.
2. Is there any uptake of fructose in the tissues? Determination of arterio-venous fructose differences.
3. The utilisation of fructose by brain.
4. Alterations in the fructose curve produced by injections of adrenalin.
5. The response to intravenous injection of fructose.
6. An investigation into the renal threshold for fructose.
7. Fructose excretion in renal disease.
8. The application of the levulose tolerance test to a /

a case of 'mixed melliturial' with which we are now in touch.

9. The levulose tolerance test in febrile subjects and

10. Simultaneous determinations of blood fructose and phosphate after the ingestion of the sugar.

11. Determinations of levulose, lactic acid, and $CO_2$-combining power of the blood in relation to the R.Q. of normal subjects during the metabolism of levulose.

12. Investigation of the levulose tolerance in cases of thyreotoxicosis. In this connection it is of interest that Cameron and Karunaratne (1936) in a study of post-mortem material from such cases found signs of liver degeneration, often to a marked degree, in a high proportion of cases, while Youmans and Warfield (1926) report disturbances of the glucose tolerance test and dye excretion test in over 50% of cases of exophthalmic goitre. The response to levulose has not before been determined.

## Bibliography.


Ashford. 1933. Biochem. J. 27:903.

Berget, Moore & Lloyd. 1932. Am. J. Physiol. 101: 565, 570.

Bertram. 1929. Zeit. ges. exp. Med. 64:295.

Bodansky. 1923. J. Biol. Chem. 56:387.

Bollmann & Mann. 1931. Am. J. Physiol. 96:683.

de Bruyn & van Eckenstein. 1896. Ber. 28:3078.

Cameron and Karunaratne. 1936. J.Path. Bact. 41:267.

Campbell & Hanna. 1926. J. Biol. Chem. 69:703.

Campbell & Maltby. 1928. J. Clin. Invest. 6:303.

Carpenter & Lee. 1933. J. Nutrition. 37:55.

Cathcart & Markowitz. 1927. J. Physiol. 63:309.

Cori. 1925. Proc. Roy. Soc. Lond. 22:495.
1925. J. Biol. Chem. 66:691.

Cori & Cori. 1928. J. Biol. Chem. 76:755.

Corley. 1929. J. Biol. Chem. 81:81.

van Creveld. 1927. Klin. Woch. 6:697.

Davidson et. al. 1936. ~~Ed. Med.~~ Biochem. J. 30:433.

Deuel. 1936. Physiol. Revs. 16:173.

Deuel & Chambers. 1935. J. Biol. Chem. 65:7.

Embden, Schmitz & Wittenberg. 1913. Z. physiol.Chem. 88:210.

Folin & Berglund. 1922. J. Biol. Chem. 51:213.

Griesbach. 1929. Z. ges. exp. Med. 65:172.

Haarman. 1932. Biochem. Zeit. 256:350.

Hansen. 1923. Acta Med. Scand.Supplementum IV.

Harding & Selby. 1931. Biochem. J. 25:1815.

Higgins. 1916. Am. J. Physiol. 41:258.


Ihl/

Ihl & Pechmann. 1885. Chem-Ztg. 9:451.

Isaac. 1913. Z. physiol. Chem. 89:78.
1920. Med. Klin. 16:1207.

Ishmori. 1913. Biochem. Zeit. 48:332.

Joliffe. 1929. J. Clin. Invest. 9:363.

Kimball. 1932. Guy's Hosp. Reps. 82:157.

Kronenberger & Radt. 1927. Biochem. Zeit. 190:161.

Laszt. 1935. Biochem. Zeit. 276:40, 44.

Laszt & Süllmann. 1935. Biochem. Zeit. 278:401.

Lohmann. 1931. Biochem. Zeit. 236:444.

McGuigan. 1908. Am. J. Physiol. 21:334.

McLean & Smedley. 1912. J. Physiol. 45:462.

Magee & Reid. 1931. J. Physiol. 73:163.

Mann. 1925. J. Am. Med. Ass. 85:1472.
Am. J. Physiol. 74:238.
Transact. Am. Ass. Physic. 40:362.

Mann & Magath. 1922. Arch. Int. Med. 30:171.

Meyerhof. 1933. Scientia. 326.

Neuberg & Leibowitz. 1928. Biochem. Zeit. 193:237.

Oppel. 1929. Biochem. Zeit. 205:47.

Paterson. 1935. Biochem. J. 29:1398.

Radt. 1928. Biochem. Zeit. 198:195.

Reinhold & Karr. 1927. J. Biol. Chem. 72:345.

Roe. 1934. J. Biol. Chem. 107:15.

Rohmann. 1915-19. Biochem. Zeit. 72:26.
do. 84:382, 399.
do. 93:237.

Rosenthal /

Rosenthal. 1922. J. Pharmacol. Exp. Therep. 19:385.

Scott. 1935. Biochem. J. 29:1012.

Seliwanoff. 1887. Ber. Chem. 1:181.

Spence & Brett. 1921. Lancet. 2:1362.

Steinberg. 1927. Pflüger Archiv. 217:686.

Stewart & Caddie. 1934. J. Physiol. 80:457.

Stewart, Scarborough & Davidson. 1937. Ed. Med.Journ. 44:105.

Tallermann. 1923. Quart. Journ. Med. 17:37.

Tashiro & Tietz. 1930. J. Biol. Chem. 87:307.

Tögel, Brezina & Durig. 1913. Biochem. Zeit. 50:296.

Verzar. 1935. Biochem. Zeit. 276:17.

Verzar & McDougall. 1936. "Absorption from the Intestine". Longmans Green & Co.

Wierznchowski. 1926. J. Biol. Chem. 68:385.

Wilbrandt & Laszt. 1932. Schweiz. Med. Woch. 1045.

Yamasaki. 1930. Biochem. Zeit. 228:123.

Youmans and Warfield. 1926. Arch. Int. Med. 37:1.